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Robert T. Frank Anniversary Number

ROBERT TILDEN FRANK

AN APPRECIATION

THE present issue of the JOURNAL possibly is an innovation in that it constitutes a memorial to a man still active in his chosen profession, who has before him a span of years when he may enjoy the fruits of his labors and the honors which have been bestowed upon him. In this country we do not always acknowledge a man's achievements du ing his lifetime nor do we afford an opportunity for his friends and colleagues to do so in a cooperative manner such as is afforded in this special issue of the JOURNAL. The Germans have a word for it, they call a volume like this a "Festschrift"—unfortunately we have no equivalent term. But its meaning and scope are clear, let us call it what we may.

This volume, as put forth, commemorates a man's career, a career which is synchronous with great advances in gynecology, advances which have altered the conception of this important branch of medicine from its previously mechanistic character to one much broader and more significant. It also presents a compliment of universal appreciation of the influence which Dr. Frank has wielded in molding present-day thought and opinion, especially in that twilight zone of endocrinology and its allied histology and pathology, in which much has been accomplished but in which much still remains to be done.

But Dr. Frank's scientific activities have not been limited to the domain of endocrinology. His work on gynecologic and obstetric pathology, first published in 1931 and since then twice revised, is an ac-

cepted and much-quoted standard. Studies dealing with the problem of uterine prolapse and its treatment, as well as that of vesicovaginal fistula, and of other plastic procedures, including the construction of an artificial vagina, constitute noteworthy additions to American gynecologic literature.

The assembled contributions to this volume represent the work of many collaborators from various American and foreign sources. Their dedication for this purpose is not merely a flattering compliment to Dr. Frank, it is an acknowledgment of that widespread and well-established interest in the diseases of woman peculiar to her sex and in the care of her reproductive processes.

The last century has been replete with great advances in the combined field of obstetrics and gynecology. The acceptance of the contagious character of puerperal fever, the establishment of the principles of antisepsis and asepsis, the clinical application of anesthesia, the extension of operative methods of approach in pelvic disease and in the dystocia of labor almost seemed to spell the end of progress at the turn of the century. But much remained in reserve for further development during the next three decades when radiation procedures for diagnosis and treatment, blood transfusions for postoperative hemorrhage, adequate and prophylactic prenatal care methods, a better understanding of the physiology and histology of menstruation and its abnormalities, and an increasing insight into the mysterious workings of the endocrine glands, constituted an era of progress which opened up new worlds of thought and activity. It is during this latter period that Robert Frank has made his noteworthy contributions in the field of endocrinology, noteworthy because they were based on careful clinical observations, supported by animal and pharmacologic experiments. The additions to our knowledge of the activities of the endocrines made by Dr. Frank and his coworkers constitute an outstanding contribution to American medical literature and represent that sane application of theoretic knowledge to clinical procedures which is often lacking but is so very important and essential.

The Editors of the JOURNAL are happy to have been afforded the opportunity to present to the medical profession these assembled contributions from outstanding clinicians and research workers, dedicated to a man who, by earnest effort and continued application, has contributed much to our knowledge in a special field of medicine. This volume and what it represents, will undoubtedly constitute a source of gratitude and satisfaction to its recipient, conveying as it does, a united expression of good will and good feeling from its numerous contributors to Dr. Frank upon his retirement merely from active service in the Mount Sinai Hospital, which he long has served so ably and so well.

—George W. Kosmak, M.D.

THE LYMPHATICS OF THE MUCOSA OF THE FIMBRIAE OF THE FALLOPIAN TUBE

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IT IS the purpose of this paper to present observations made in the endeavor to ascertain the distribution of the lymph vessels in the mucosa of the fimbriae of the fallopian tube and their relation to the lymphatics of the ampulla of the tube and to those of the mesosalpinx and the ovary.

In the study of carcinoma of the tubal mucosa secondary to carcinoma of the ovary, I have been impressed both with the frequency of the location of these secondary tumors in the fimbriae of the tube and also, in some of these cases, with the presence of the growth in spaces which I believed might be lymph vessels of the mucosa of the fimbriae. In attempting to determine the pathogenesis of these secondary tumors I have been handicapped by a lack of any conception not only of the distribution of the lymphatics in the mucosa of the tubal fimbriae but also of their relation to the lymphatics of the ampulla of the tube and those of the mesosalpinx and the ovary.

Andersen[1] (1927) referred to the contributions of Poirier (1889-90), Bruhns (1898), Bartels (1909), and Poirier and Cuneo (1920) indicating that the lymph vessels from the tube empty into the subovarian plexus and there unite with lymph vessels from the ovary and the uterus. Andersen has shown that in the sow (Fig. 1 of her paper) the lymph vessels from all portions of the tube, including the fimbriae, converge to form the subovarian plexus which also receives lymph vessels from the ovary and the uterus.

Pellé and Pellé[2] (1931) by first injecting the lymphatics of the tubes with Paris blue and then those of the ovary or uterus with yellow of cadmium observed that the blue-colored lymph vessels, coming from the tubes, soon became green, thus indicating an early communication between the lymph vessels of the tube and those of the ovary and the uterus. They believe that their experiments verify the opinions expressed by Bruhns and by Bartels to which they refer in their paper. These anatomic observations may be further confirmed by the abundant evidence that carcinoma may spread from either the uterus or the ovary to the tube by way of the lymph vessels.

Little apparently is known about the lymphatics in the tubes of women and least of all about those in the fimbriae.

Andersen[1] states: "The existence of lymphatics in the fallopian tube has been suspected for a long time by the students of the histology of the tube. They have noted only the lymphatics of the mucosal folds of the ampulla." In a historical

Fig. 1.—Cross-section of the ampulla of the fallopian tube of the sow showing the injected lymphatics (from Andersen[1]). The most conspicuous lymph plexus is situated in the mucosa. The vessels in this plexus are typical lymph capillaries without valves. Those in the mucosal folds empty into similar vessels at the base of the folds. Vessels from the latter pierce the inner muscular layer and empty into valved collecting vessels situated between the two muscular layers. These vessels in turn empty into lymph vessels in the mesosalpinx. The subserous plexus is less conspicuous and consists of small capillaries just outside the outer muscular layer, separated from the serosal mesothelium by a few strands of connective tissue. This plexus also drains into the intermuscular vessels. ×56.

Fig. 2.—Photomicrograph of a portion of an oblique section of the ampulla of a normal appearing tube. The patient, A. H. No. 1920-22, parous, aged forty-one, had had the entire uterus and one tube and ovary removed for a primary uterine endometriosis. Irregular-shaped channels or spaces lined by endothelium-like cells are present in some of the folds and also at the base of the folds. The form and situation of these spaces are similar to those of the injected lymphatics of the mucosa shown in the preceding illustration. I believe they are lymph vessels. Compare also with the next photomicrograph. ×25.

Fig. 3.—Cross-section of a portion of the ampulla of the left tube in which the lymphatics of a large mucosal fold are filled, as with an injection mass, with carcinoma secondary to that in the ovaries. The patient, A. H. No. 9895, parous, aged forty-seven, had had both tubes and ovaries and the uterus removed for bilateral ovarian carcinoma associated with an extensive peritoneal carcinomatosis. Carcinoma was found in lymphatics in all layers of this tube and also in lymph vessels in the mesosalpinx. The form and situation of the carcinoma-filled lymph vessels in this fold and also in the mucosa at the base of the fold (''*a*'') are very similar to the injected lymph vessels shown in Fig. 1 and also to the judged lymph vessels shown in Fig. 2. The condition present in this section gives added support to the belief that the empty spaces shown in the mucosa of Fig. 2 are lymph vessels. ×25.

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review of the lymphatics in the tubal mucosa, Andersen mentions the observations of Henle (1873), Orthmann (1887), Sobotta (1903), Hörmann (1908), Grosser (1919), and Graf Spee (1924). She also states: ''A number of workers on the lymphatics of the uterus have likewise discovered the presence of lymphatics in the tube.''

Hörmann[3] (1908) evidently observed lymph vessels in the mucosa of the fimbriae as well as in the mucosa of the ampulla of the tube. He frequently found large irregular empty spaces in the large mucosal folds of the ampulla and of the fimbriae which he stated had attracted the attention of earlier writers. Hörmann in his paper gave Henle's and Orthmann's descriptions of these spaces. He was first inclined to regard these spaces as artefacts but, after observing that they occurred with great regularity both in form and distribution in all of his preparations and that in many places they appeared to be lined by endothelium, he concluded that although definite proof was lacking, these spaces were most likely lymphatics.

Andersen[1] writes that she has been ''able to discover in an earnest perusal of the literature . . . no description whatsoever of the pattern of the lymph vessels within the tubal wall.'' The absence in the literature of a description of the intrinsic lymphatics of the tube is due to the difficulties encountered in injecting these vessels.

Andersen[1] injected the lymphatics of the tubes of sows. She was able to obtain injections of the lymphatics of all portions of the ampulla of the tube and of the tubouterine junction in that animal. Due to technical difficulties she was unable to inject the lymphatics of the mucosa of the isthmus of the tube and of the infundibulum. She believed, however, that the infundibulum does contain a lymph capillary plexus as yet not demonstrated.

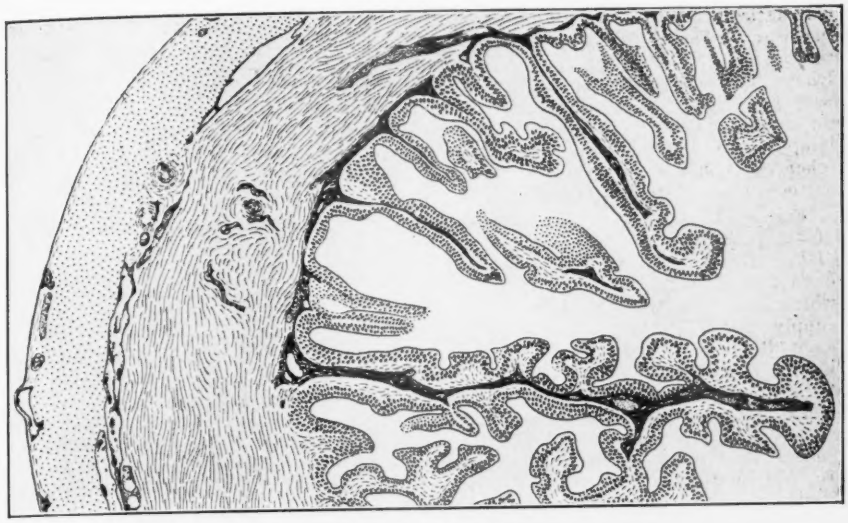

Fig. 1.

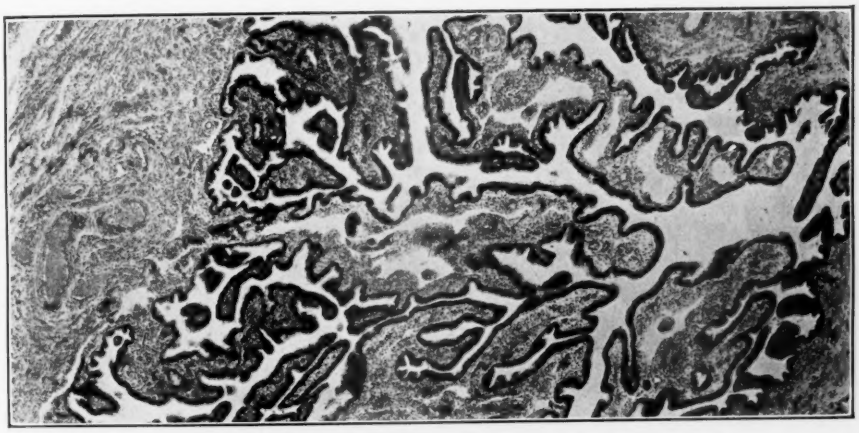

Fig. 2.

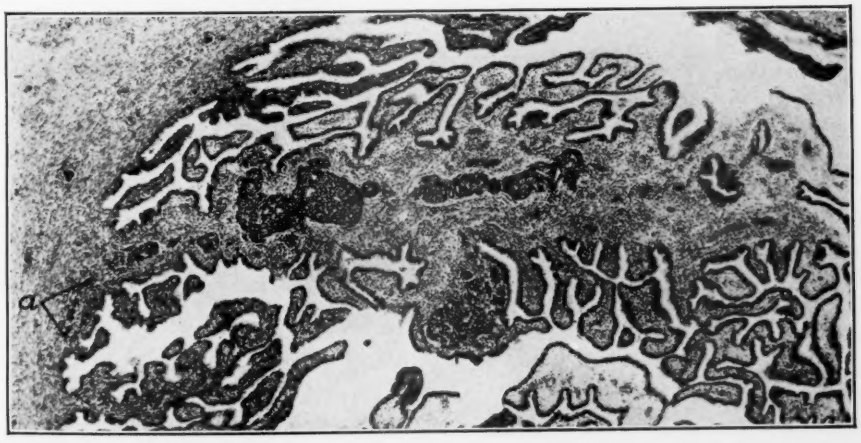

Fig. 3.

Fig. 4.—Cross-section of a portion of the ampulla of the left tube in which the lymphatics are filled, as with an injection mass, with carcinoma secondary to that of the ovary. The patient, A. H. No. 93646, nulliparous, aged forty-six, had had both tubes and ovaries and the uterus removed for a large carcinoma of the left ovary. No evidence of peritoneal carcinomatosis was observed at the operation. Compare with Figs. 1, 2, and 3. The distribution of the lymph vessels of the mucosa both in the folds and at their bases is similar in all four illustrations. By noting the lymphatics in each mucosal fold of this section and superimposing the folds on each other one can visualize the distribution of the lymphatics as seen in cross-sections of these mucosal folds. ×54.

Fig. 5.—Cross-section of the ampulla of the tube in which lymphatics in all layers of the tube are filled, as with an injection mass, with carcinoma secondary to that of the uterus. The patient, A. H. No. 4727-31, parous, aged sixty-four, was operated upon for uncontrollable bleeding due to an advanced carcinoma of the uterine cervix which had been treated with radium. An attempt was made to ligate the blood supply of the uterus. The retroperitoneal tissues on both sides of the pelvis were so infiltrated by the growth that it was impossible to gain access to the anterior branch of either internal iliac artery. The right tube and ovary were removed and the left ovarian vessels and both round ligaments were ligated. The bleeding ceased and did not return. The patient died a year later. The study of the tube and ovary removed at operation indicated that the carcinoma had extended from the uterine cornu into the lymph vessels of the mesosalpinx and thence to the ovary and tube. In this section the mucosal lymphatics greatly distended with carcinoma are most striking. Compare with the subserosal lymph vessels which are few in number if their injection is complete. Lymph vessels in the tubal wall are also filled with carcinoma. The carcinoma-filled vessel marked "*a*" is a collecting lymph vessel extending from the tube into the mesosalpinx at the right. ×16.

The human fimbrial mucosa consists of a continuation of the mucosa of the ampulla with its longitudinal folds through and beyond the abdominal ostium of the tube. This mucosa spreads out and lines the inner surface of the infundibulum of the tube, adapting itself to the size and shape of the latter. It also extends over the rim of the infundibulum and covers the outer surface of the latter for a varying distance to join the serosa of the tube. A further extension of this mucosa along the free margin of the mesosalpinx, toward or to the tubal pole of the ovary, constitutes the mucosa of the ovarian fimbriae.

By floating out the fimbriae of a freshly removed tube in water and by examining it with a hand lens one will be impressed not only with the great variation in the size and shape of the mucosal folds but also with the complexity of their arrangement. If a similar examination is made of the same tube after incising it longitudinally one may see that the arrangement of the fimbrial folds becomes less complex and that the continuity of many of these folds with the longitudinal folds of the ampulla is evident. A comparative examination of several fimbriae demonstrates a great variation in the pattern of the mucosal folds in the different fimbriae. Since the histologic structure of the fimbrial and ampullar mucosa is essentially the same one might infer that the distribution of the lymphatics in the two situations would be similar. A knowledge of the distribution of the lymphatics in the mucosa of the distal portion of the ampulla would be of great assistance in the study of the unknown distribution of the lymphatics in the fimbrial mucosa.

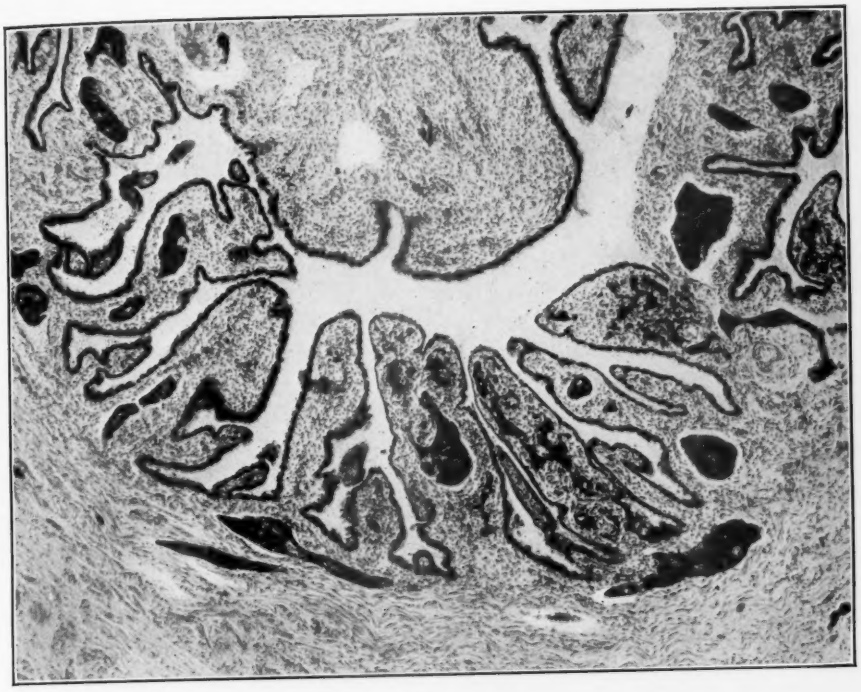

Fig. 4.

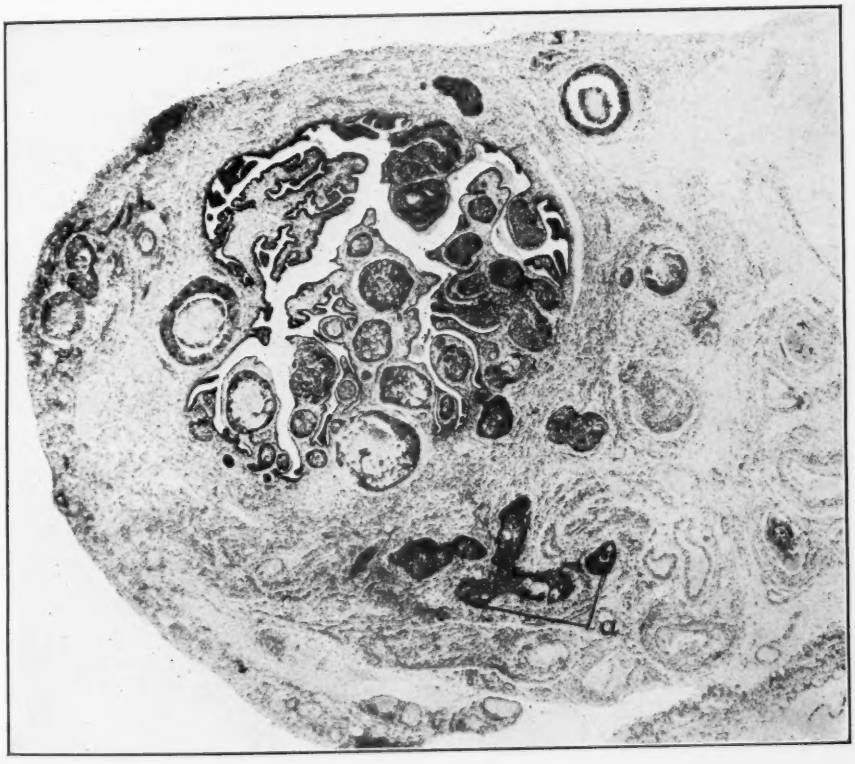

Fig. 5.

Fig. 6.—Longitudinal section of the distal portion of the tube, shown in the preceding illustration, including the fimbriae proper and the free margin of the mesosalpinx with its ovarian fimbriae, at the right, cut obliquely. As in the preceding section, the lymphatics of the mucosa are distended with carcinoma. Many of these are cut either longitudinally or obliquely and are thus extending lengthwise in the tubal mucosa. Note that the fimbrial mucosa is continuous with the mucosa of the ampulla and also that the lymphatics in the mucosa in the two situations appear to be continuous. Large lymph vessels distended with carcinoma are present in the wall of the tube and also in the mesosalpinx beneath the ovarian fimbriae. Apparently the lymph vessels of the ovarian fimbriae drain into vessels in the mesosalpinx beneath. ×5.

Fig. 7.—Higher magnification of a portion of the fimbriae shown in the preceding illustration. The distribution of the lymph vessels in the mucosal folds and at their bases is similar to that shown in the mucosa of the ampulla of this and other tubes (compare with Figs. 1, 2, 3, 4, and 5). ×25.

I have used Andersen's[1] excellent description of the injected lymphatics of the ampulla of the sow's tube as a guide in the study of the lymphatics of the mucosa of the ampulla of human tubes.

"The lymphatics of the ampulla lie in three separate strata of tissue. The two capillary networks lie in the subserous and subepithelial connective tissue respectively, and the large collecting vessels lie in the intermuscular connective-tissue layer. . . .

"In cross-section the most conspicuous plexus is the mucosal one (Fig. 1 of the present paper). The vessels are broad in the long axis of the tube and thin in the transverse axis. They enter each mucosal fold, running approximately perpendicular to the inner surface of the circular muscle, so that if a cross-section cuts through a vessel near the base of the fold it is likely to cut lengthwise through a considerable portion of it. As the lymph-vessel extends farther it divides into two approximately equal branches, usually at an angle of 60 degrees or over, and repeats this method of branching at fairly regular intervals as it continues into the fold. These branches run in all directions through the mucosal folds, but the majority of them run longitudinally with the tube. . . . The vessels are typical lymph capillaries, without valves and with the great irregularity of lymph vessels. . . .

"The lymphatic network of one mucosal fold is connected with that of the next by direct continuity at the base of the folds. This network bears the same relation to the epithelium between folds as to that of the folds proper. At the base of each fold the lymph-vessels widen into small sinuses as they receive the vessels from either side. Similar smaller sinuses are often found at the base of secondary folds. The plexus of vessels in the mucosa thus consists of a rich network just beneath the circular muscle, which receives lymph from the plexus in the mucosal folds. It drains into the intermuscular collecting vessels by way of fairly frequent vessels which pierce the circular muscle, usually in company with arterioles (Fig. 1 of the present paper).

"The lymph-vessels lie in the connective tissue of the mucosal fold. They are separated from the nearest epithelial cells by only a few strands of connective tissue, while they may be a considerable distance, often as much as 50 microns, from other epithelial cells. . . .

"The subserous plexus is less rich, and consists of small capillaries just outside the longitudinal muscle-layer and separated from the serous mesothelium by a few strands of connective tissue. This plexus also drains into large intermuscular vessels. . . .

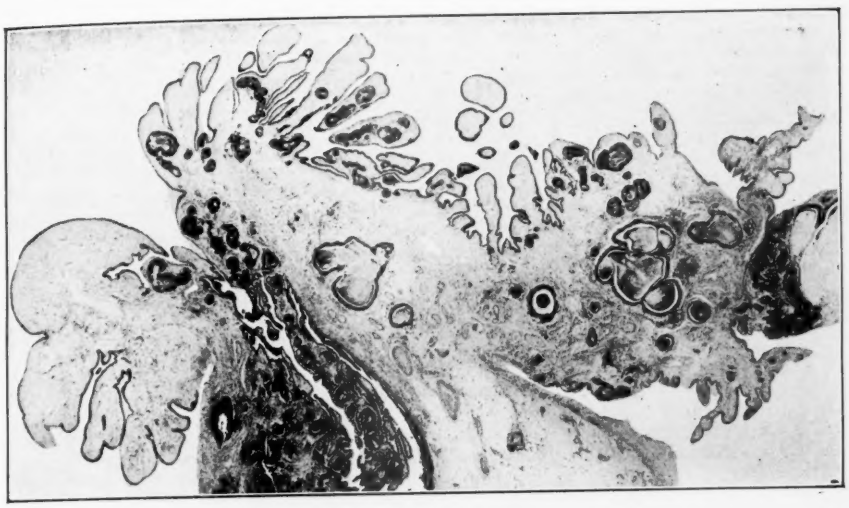

Fig. 6.

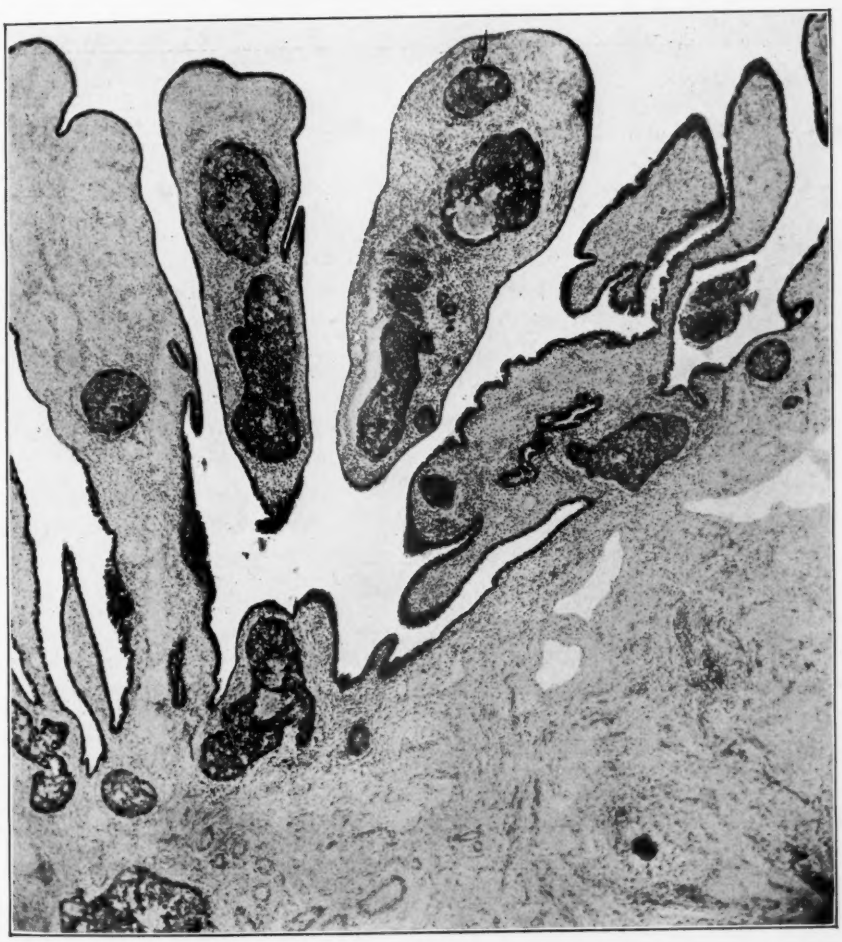

Fig. 7.

Fig. 8.—Longitudinal section of the distal portion of the tube including its fimbriae proper, the mesosalpinx with its ovarian fimbriae and the tubal pole of the ovary at the right. The patient, A. H. No. 9339-31, parous, aged forty-seven, had had the entire uterus and one tube and ovary removed for a uterine leiomyoma. The veins filled with blood appear black in the photomicrograph. Their distribution suggests an intimate relationship between venous outlets of the tubal pole of the ovary, the fimbriae, and the mucosa of the distal portion of the tube. The large irregular empty space "*a*" arose from an incomplete section of the mesosalpinx. I believe that spaces "*b*," "*c*," and "*d*" are the lumina of large lymph vessels. There is no suggestion of an anastomosis between the lymphatics of the ovarian fimbriae and those of the ovary. Occasional lymph vessels can be seen, under higher magnification, in the compressed folds of the mucosa of the ampulla. On the other hand, dilated lymphatics can be easily seen in many of the folds of the fimbrial mucosa, especially in the folds at the left of the abdominal ostium of the tube. It is obvious even with this low magnification that the fimbrial mucosa is but a continuation of the mucosa of the ampulla beyond the ostium of the tube. ×5.

Fig. 9.—Higher magnification of the fimbrial mucosa about the abdominal ostium of the tube shown in the preceding photomicrograph. The extension of the longitudinal folds of the mucosa of the ampulla through and beyond the ostium of the tube can be easily seen, especially at the left. Lymph vessels running longitudinally in these folds can also be detected. These vessels become more dilated and are therefore more evident the further the mucosal folds extend beyond the ostium. The distribution of the lymph vessels in the folds of the fimbrial mucosa and at their bases is similar to that of the lymph vessels of the mucosal folds of the ampulla shown in this photomicrograph and better pictured in Figs. 1, 2, 3, and 4. ×25.

"The collecting vessels, which are valved, run in the connective tissue which lies between the two muscle-layers." These vessels pass through the tubal wall and empty into the lymph vessels of the mesosalpinx.

METHODS OF STUDY

Although I have failed in all of the few attempts made to inject the lymphatics of the tube, I have, however, observed, in the mucosa of the ampulla of tubes from patients with carcinoma of either the ovary or the uterus, spaces which were filled with carcinoma as with an injection mass. I inferred that these carcinoma-filled spaces were lymphatics. I have also frequently seen irregular, empty spaces in other portions of the tubal mucosa just described and also in the mucosa of normal tubes which, in form and situation, resembled the spaces filled with carcinoma. In these empty spaces an endothelium-like lining could be detected. I gathered this material together and compared the spaces just described with Andersen's[1] illustration and description of the injected lymphatics of the mucosa of the ampulla of the sow's tube. I found that the judged lymphatics observed by me were very similar, both in form and distribution, to the injected lymphatics shown by Andersen (see Figs. 1 to 6). It would seem that Andersen's description of the lymphatics of the mucosa of the ampulla of the sow's tube might at least in a general way be applied to the lymphatics of the mucosa of the ampulla of the human tube.

Realizing that the mucosa of the fimbriae is a continuation of the mucosa of the ampulla through the abdominal ostium of the tube and

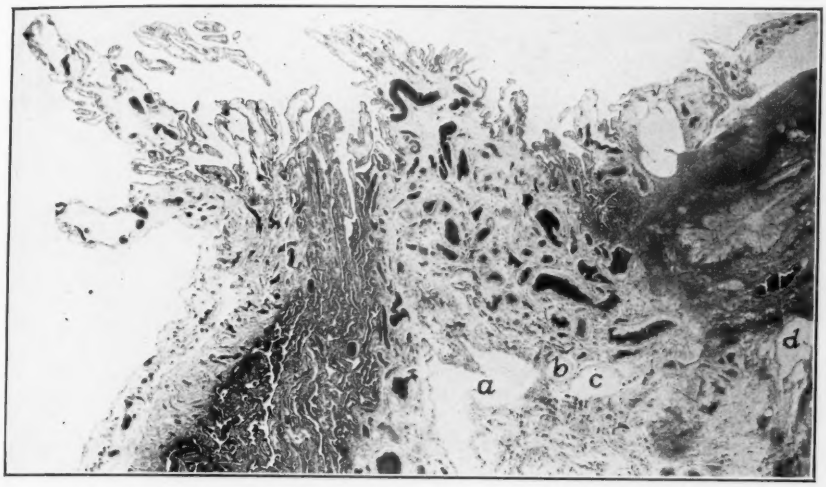

Fig. 8.

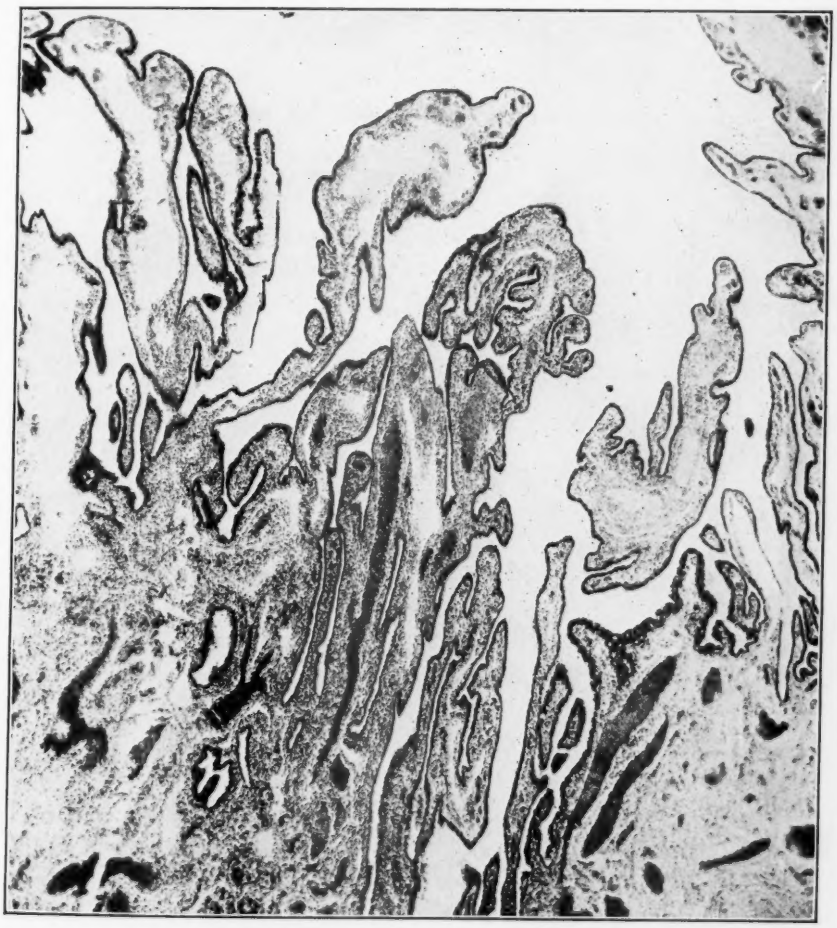

Fig. 9.

Fig. 10.—Longitudinal section of the distal portion of the tube including the fimbriae, in a plane at right angles to that shown in Fig. 8. The patient, A. H. No. 7788-31, parous, aged thirty-nine, had had the entire uterus and one tube and ovary removed and the pelvic floor repaired for descensus of the uterus and other results of the injuries of childbirth. The veins filled with blood are easily discernible. The lymph vessels cannot be detected in the compressed folds of the mucosa of the ampulla. On the other hand, they are evident in many of the mucosal folds of the fimbriae. A mucoserosal junction is indicated by the letter "*a*." ×10.

Fig. 11.—Higher magnification of mucosal folds of the fimbriae shown in the lower right-hand portion of the preceding photomicrograph. The lymph vessels in the folds and at their bases can be easily seen. The distribution of these vessels and the ones in the folds of the ampulla, shown in Figs. 1, 2, and 3, is similar. Although lymph vessels for the most part lie in the central portion of the folds, their distance from the epithelium varies in different portions of the folds. Lymph vessels "*a*," "*b*," and "*c*" accompanying blood vessels can be seen in the wall of the tube. If these vessels are continuous with those at the base of the folds, as well they may be, they would furnish an outlet for the lymph in the mucosal lymphatics. ×54.

Fig. 12.—Higher magnification of mucosal folds of the fimbriae at the right of the ostium of the tube shown in Fig. 8. The lymph vessels in the folds and at their bases can be easily seen. The distribution of these vessels and the ones in the preceding photomicrograph is similar. In both instances the tubes were cut longitudinally but in planes at right angles to each other. Note that the blood vessels in the folds are situated, for the most part, between the lymph vessels and the epithelium. This apparently is a consistent arrangement. ×25.

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that the mucosa in the two situations has the same histologic structure and therefore should have a similar lymphatic distribution, I studied carcinoma-filled and empty lymphatics of the fimbrial mucosa by comparing them with similar lymphatics in the mucosa of the ampulla.

In order to trace the extension of the mucosa of the ampulla of the tube into the fimbriae fifty-nine normal appearing tubes were studied. The tubes and ovaries attached to uteri which had been removed at operation were severed from these uteri and immediately placed in 10 per cent formalin without incising the tubes. The distal portion of these tubes, including their fimbriae, was imbedded in celloidin since this causes less unequal tissue shrinkage and therefore fewer artifacts than paraffin. The sections were stained with hematoxylin and eosin.

Sections cut in three different planes were employed in the study of these fifty-nine specimens. In one series the distal portion of the ampulla including its fimbriae, the mesosalpinx with its ovarian fimbriae and a small portion of the tubal pole of the ovary were so mounted that longitudinal sections would include all of these structures. In the second series the distal portion of the tube and its fimbriae were cut longitudinally in planes at right angles to those of the first series. In the third series cross-sections were made of the fimbriae and distal portion of the tube. Serial sections were not employed in this group of specimens. However, representative sections from different levels of each block were saved. Lymph vessels could be seen in the mucosa of the fimbriae in all of the sections.

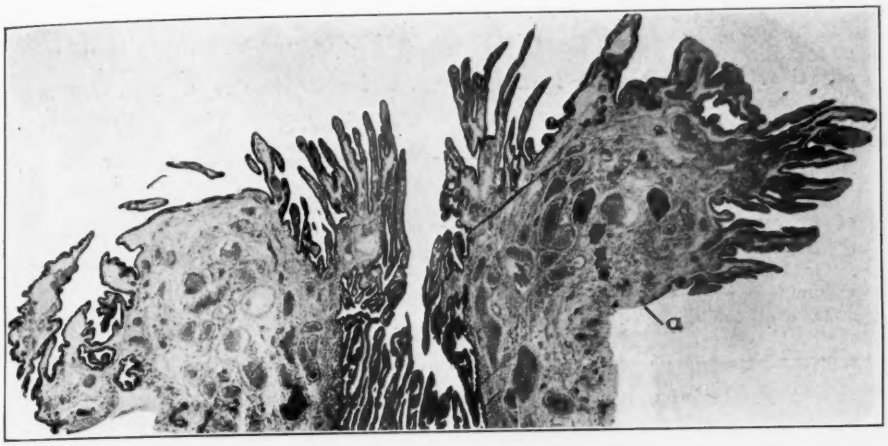

Fig. 10.

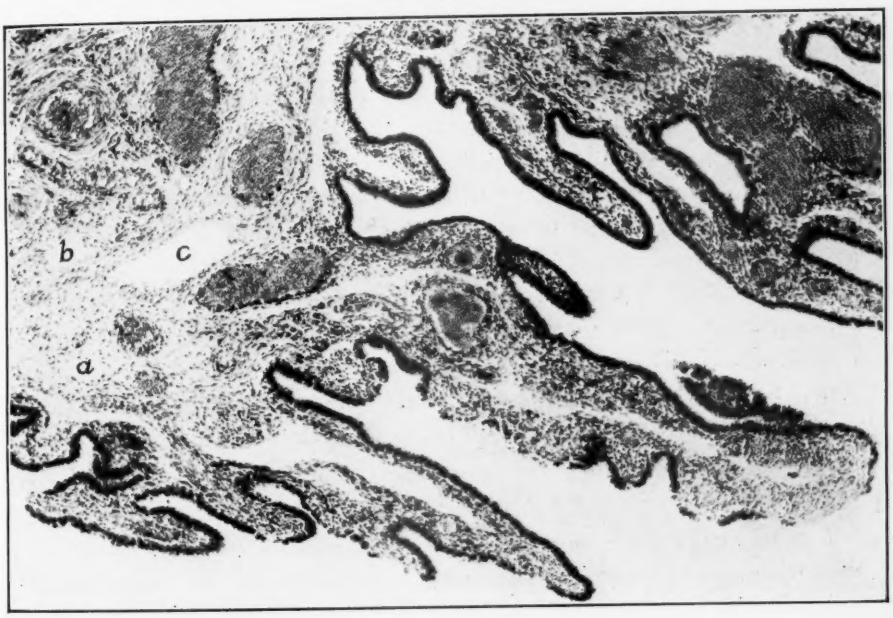

Fig. 11.

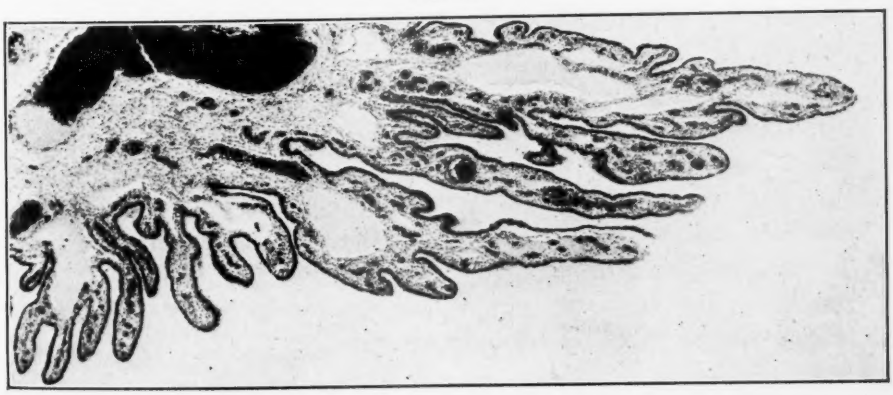

Fig. 12.

Fig. 13.—Mucosal folds of the fimbriae and a portion of the ampulla at the left of the ostium of the tube shown in Fig. 10, from another section. A portion of the ostium of the tube appears at the right in this photomicrograph. Note that the lymph vessels of the folds empty into vessels at the base of the folds. The latter apparently are continuous with lymph vessels of the mucosa of the ampulla and also possibly with lymph vessels "*a*" and "*b*" (more evident in lymph vessel "*b*") beneath the fimbrial mucosa. ×54.

Fig. 14.—Mucosal folds of the fimbriae and a portion of the ampulla at the right of the ostium of the tube shown in the preceding photomicrograph, from the same section. A portion of the ostium of the tube appears at the left in this photomicrograph. Note the lymph vessels in the folds and at the base of the folds. The vessel in the fold nearest the ostium apparently is continuous with the lymphatics of the mucosa of the ampulla. A mucoserosal junction appears in this photomicrograph at "*a.*" There is no evidence of any anastomosis between the mucosal and the subserosal lymphatics. The latter are not evident. ×50.

In a smaller group of five specimens the tubes were incised longitudinally before placing them in formalin. This was done in order that the mucosa of the ampulla would not be compressed by the shrinkage of the wall of the tube in the fixing solution as occurred in the first group. Sections from this group showed no compression of the mucosa of the ampulla with a resulting increased visibility of its lymph vessels. Complete serial sections were employed in a portion of the blocks in all of these specimens. I have found that it is possible to follow a noninjected lymph vessel for only a short distance even in complete serial sections because portions of the vessel may be constricted and therefore difficult or impossible to detect.

The lymphatics seen in the various types of mucosal folds were compared with those seen in similar types of mucosal folds in the same and other sections. In this way one obtained a general impression of the distribution of the lymphatics in each type of mucosal fold.

Noninjected lymph vessels may be confused wth empty veins and also with spaces caused by unequal tissue shrinkage. Fortunately the veins in my preparations usually contain blood. Artifacts are infrequent and are usually easily recognized both by their appearance and by the fact that they are not lined by endothelium-like cells.

The photomicrographs with their legends present my observations and interpretations better than any written description. These constitute the most important part of this paper. They also demonstrate both the value and the shortcomings of this method of study. I desire others to draw their own conclusions not only from a study of the photomicrographs in this paper, but of much greater importance, from the study of similar material of their own in which, if possible, the lymphatics have been injected. I have found a reading glass of great value in the study of the photomicrographs.

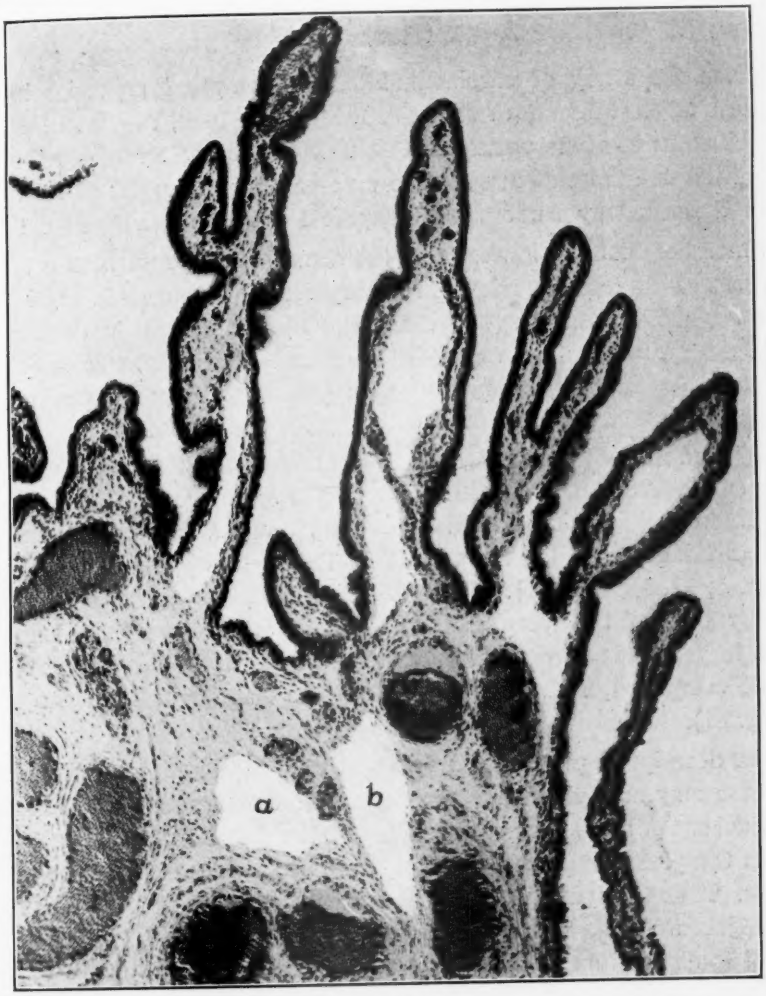

Fig. 13.

Fig. 14.

DISCUSSION

From the study of carcinoma-filled and empty lymphatics in the mucosa of the ampulla of the human fallopian tube, I believe that the distribution of these vessels in this mucosa closely resembles the distribution of the lymphatics in the mucosa of the ampulla of the sow's tube described by Andersen[1] (compare Figs. 2 to 6 with Fig. 1).

Since the fimbrial mucosa has the same histologic structure as the mucosa of the distal portion of the ampulla with which it is continuous one might infer that the distribution of the lymphatics in the mucosa in these two situations would be the same. This inference is correct as indicated in the photomicrographs (see Fig. 9 and compare Fig. 4 with Fig. 7, and Figs. 2 and 3 with Figs. 11, 12, and 13).

By comparing the lymph vessels in mucosal folds of approximately the same size and shape in one or in several sections of both the ampullar and fimbrial mucosa one is able, in a general way, to visualize the distribution of the lymphatics in that type of mucosal fold (see Figs. 4, 11, 12, and 20). Since mucosal folds vary in size and form the pattern of the lymphatics in these folds must vary accordingly. The larger folds with secondary folds arising from them will have a more complex lymphatic pattern than the smaller and simpler folds (Fig. 15).

For descriptive purposes the lymphatics of the ampullar and fimbrial mucosa may be divided into two plexuses: one situated in the mucosa at the base of and between the folds and the other in the folds. Vessels from the plexus in the folds empty into the plexus at the base of the folds. Thus the lymphatics of one mucosal fold are united with those of adjacent folds. This pattern prevails in all sizes and types of mucosal folds whether in the ampulla or fimbriae.

I have been unable to ascertain either the pattern of the branching and anastomosing of the lymph capillaries in the folds or the form of their termination (really origin) in the crest of the folds, whether the latter occurs in blind ends or loops. Only by a careful study of tubes in which the lymphatics have been injected can these finer and interesting details be determined. In the sections of the fimbriae studied by me the lymph vessels of the mucosa were usually more dilated and therefore more easily seen than the vessels in the ampullar mucosa of the same tube. In spite of this fact I experienced almost as much difficulty in tracing, even in serial sections, these noninjected capillaries in the fimbrial folds as I did in the ampullar folds.

Since the lymphatics of the ampullar and fimbrial mucosa are true capillaries without valves a free circulation of the lymph in all directions in the plexuses is assured.

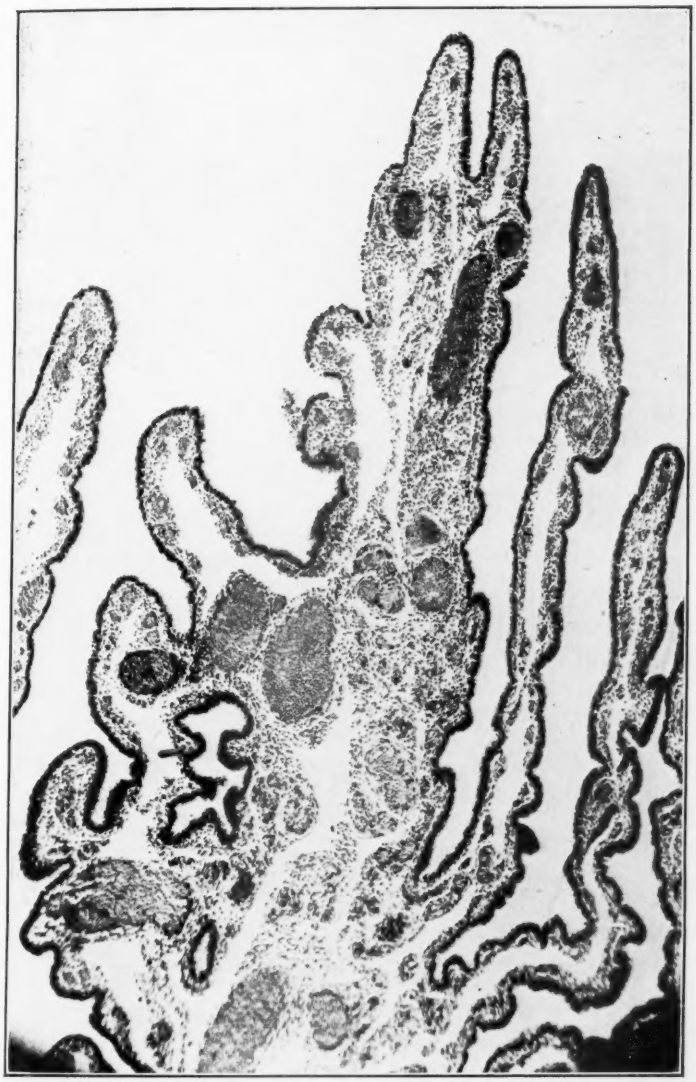

Fig. 15.—A large primary mucosal fold of the fimbriae with many secondary folds, from the section shown in the two preceding photomicrographs. Note the complexity of the pattern of the lymph vessels in the large fold and its relative simplicity in the secondary folds. The vessels in the secondary folds empty at their base into the lymph vessels of the large fold just as the lymph vessels of a primary fold drain into the lymph vessels at its base. ×50.

Fig. 16.—Cross-section of the fimbriae of a normal appearing tube at the level of its abdominal ostium (see Fig. 10 as a guide for the plane of this section). The patient, A. H. No. 970-32, parous, aged forty-nine, had had the uterus removed for benign uterine bleeding and descensus of that organ. The pelvic floor was also repaired for the results of the injuries of childbirth. The mucosa of the fimbriae covers over two-thirds of the surface of the circumference of the tube in this section. The lymph vessels of the mucosal folds of the fimbriae are greatly dilated as are those of the fimbriae shown in Fig. 19. ×5.

Fig. 17.—Higher magnification of the fimbria in the upper right-hand portion of the section shown in the preceding photomicrograph. The veins, partly or completely filled with blood which stains darkly, can be easily seen. I believe that all of the empty spaces are the lumina of lymph vessels. By using a reading glass one can easily see the lymphatics, at the base of and between the folds, which drain the greatly dilated lymphatics of the folds and in turn empty into vessels in the stroma of the fimbria proper beneath them. ×25.

Fig. 18.—Higher magnification of the fimbrial mucosa at its junction "*a*" with the serosa appearing in the left-hand portion of the section shown in Fig. 16. Note the dilated lymphatic, beneath the epithelium, which in this section appears to end blindly with the termination of the mucosa. In the examination of numerous sections, from many tubes, I have been unable to observe an anastomosis between the mucosal and the subserosal lymphatics. On the other hand, I have observed, in many sections, indications that the lymphatics of the terminal portion of the fimbrial mucosa are drained by vessels penetrating the tubal wall. ×54.

There is abundant evidence that the lymphatics at the base of and between the mucosal folds of the fimbriae about the ostium of the tube are continuous with similar lymphatics of the mucosa of the distal portion of the ampulla (see Figs. 6, 9, 13, and 14). Also when a fimbrial mucosal fold is a continuation of a longitudinal fold of the ampulla the lymphatics in the folds as well as at their bases should be continuous (Fig. 9). Therefore some of the drainage of the lymphatics of the fimbrial mucosa must be through the lymphatics of the mucosa of the ampulla.

There are indications that the mucosal lymphatics of the fimbriae drain into vessels in the wall of the infundibulum and also in the mesosalpinx beneath this mucosa just as the lymphatics of the ampullar mucosa drain into vessels penetrating the wall of the tube beneath it.

I have not been able to detect any evidence of an anastomosis between the lymphatics of the fimbrial mucosa and the subserosal lymph vessels at the mucoserosal junction even in fimbriae in which the mucosal lymph vessels were filled with carcinoma (see Figs. 18, 20, and 21). Subserosal lymph vessels in this situation could not be detected in any of my sections. This may have been due to the normal scarcity of lymphatics beneath the serosa of the distal portion of the ampulla.

In only one instance could I detect any suggestion of the possibility of an anastomosis between the lymph vessels of the ovarian fimbriae

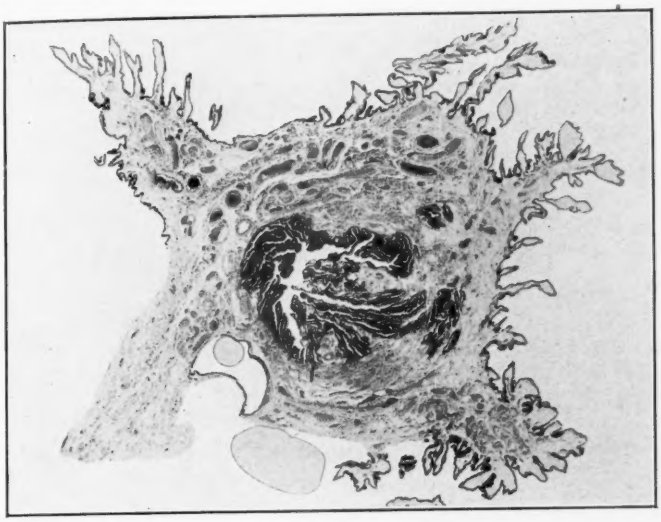

Fig. 16.

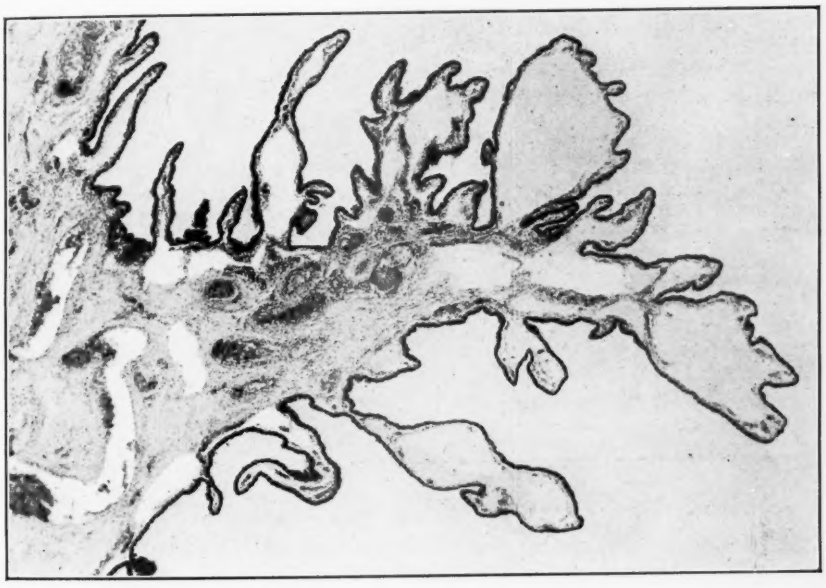

Fig. 17.

Fig. 18.

Fig. 19.—Longitudinal section of the distal portion of a normal appearing tube, including its fimbriae proper, the free margin of the mesosalpinx with its ovarian fimbriae and a small portion of the ovary at the right. The patient, A. H. No. 8389-31, parous, aged forty-three, had had the entire uterus and one tube and ovary removed for multiple uterine leiomyomas. Lymphatics cannot be detected in the compressed mucosal folds of the ampulla. On the other hand the lymph vessels in nearly all of the mucosal folds of the fimbriae are greatly dilated and are therefore conspicuous. Note the great variation in the form and height of the mucosal folds. An anastomosis between the lymph plexus at the base of the folds of the ovarian fimbriae and the ovarian lymphatics cannot be detected. The latter are not evident. ×5.

Fig. 20.—Higher magnification of mucosal folds of the fimbria in the left-hand portion of the section shown in the preceding photomicrograph. The lymphatics of the mucosa are easily seen. They do not extend beyond the mucoserosal junction (at the right). There is no evidence of any anastomosis between them and those of the subserosa. The latter lymphatics are not evident. The judged lymph vessels "*a*" and "*b*" in the tube possibly may drain the nearby mucosal lymphatics. ×54.

Fig. 21.—Longitudinal section of a portion of the fimbriae of a tube with the mucosal lymph vessels filled with carcinoma as with an injection mass. The patient, A. H. No. 113036, parous, aged forty-nine, had had both tubes and ovaries and the uterus removed for carcinoma of both ovaries associated with an extensive peritoneal carcinomatosis. The carcinoma in this section is, for the most part, confined to the mucosal lymphatics. In places, however, it has extended beyond these vessels and invaded the tissues of the folds. Carcinoma in lymph vessels of the stroma of the infundibulum is indicated by the pointer "*a*." A mucoserosal junction is indicated by the letter "*b*." Note that carcinoma is present in the mucosal lymphatics almost up to the mucoserosal junction. The subserosal lymphatics are not evident. If carcinoma were present in the subserosal lymphatics in this situation it would indicate or at least suggest an anastomosis between them and the mucosal lymph vessels. I have never found carcinoma in the subserosal lymphatics in other specimens similar to this one. Note that the pattern of the carcinoma-filled lymph vessels of the mucosa near its junction with the serosa is similar to that shown in the preceding photomicrograph. Many blood vessels are present in the tissues of the infundibulum but very few recognizable lymph vessels. The observations presented in this paper form a chapter in the study of carcinoma of the tubal mucosa secondary to carcinoma of the ovary. Is lymphatic permeation and embolic metastasis by way of the lymph vessels from the ovarian tumor the only way that the carcinoma in the ovary can gain access to the mucosal lymph vessels of the tubal fimbriae? I hope to answer this question in a later paper. ×10.

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and lymph vessels coming from the hilum of the tubal pole of the ovary. In sections of this specimen judged lymph vessels accompanying blood vessels could be seen coming from the ovary into the tissues of the mesosalpinx beneath the ovarian fimbriae and also judged lymph vessels extending from the ovarian fimbriae toward the ovarian lymph vessels. Whether or not an anastomosis occurred between these two sets of lymph vessels could not be determined.

It would seem that lymph plexuses as rich and apparently as purposeful as those present in the fimbrial and ampullar mucosa must have some important function other than to furnish channels in which carcinoma may grow and spread.

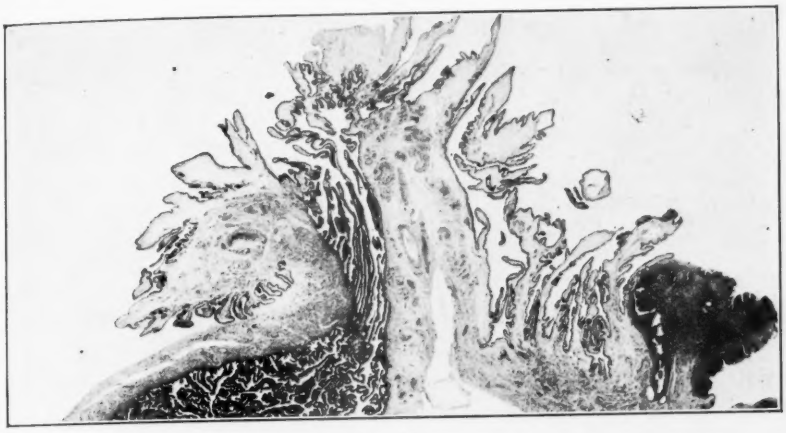

Fig. 19.

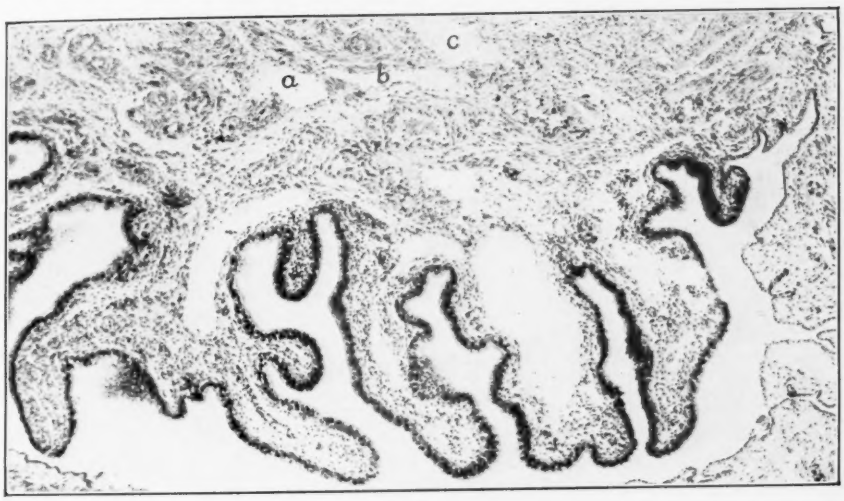

Fig. 20.

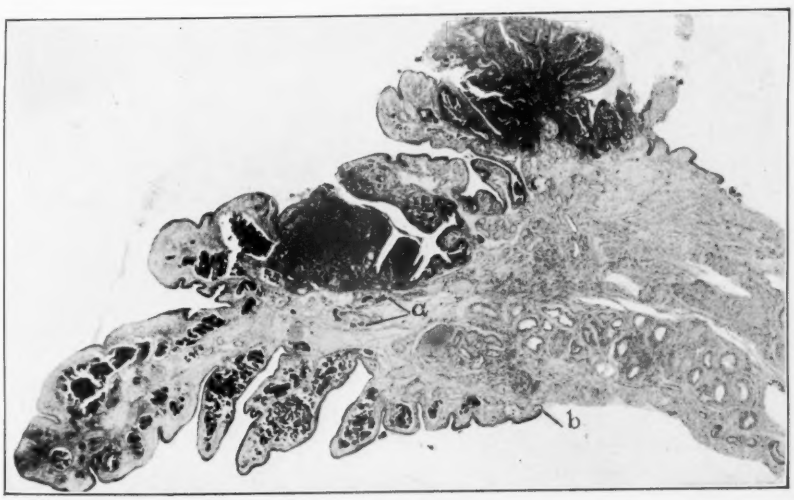

Fig. 21.

CONCLUSIONS

The mucosa of the fimbriae of the fallopian tube is richly supplied with lymphatics which are continuous with similar vessels in the mucosa of the distal portion of the ampulla of the tube.

The lymphatics of the fimbrial mucosa also drain into lymph vessels in the wall of the infundibulum of the tube and those of the ovarian fimbriae drain into lymph vessels in the mesosalpinx.

An anastomosis between lymph vessels, coming from the hilum of the tubal pole of the ovary, and the lymphatics of the adjacent ovarian fimbriae may well exist but was not positively demonstrated.

No suggestion was found of an anastomosis between the mucosal lymphatics of the fimbriae and the subserosal lymphatics at the mucoserosal junction.

NOTE: The efficiency of the laboratory work in the preparation of this paper is, in large measure, due to the technical skill and interest of Miss Helen Buchan and Miss Winifred Lansing. The photomicrographs were made by Mr. James A. Glenn. These I thank for their interest and cooperation.

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THE DEMONSTRATION OF GONADOTROPIC SUBSTANCES IN THE BLOOD AND URINE

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AMONG the many medical contributions of Dr. Robert Tilden Frank, one of the most outstanding is the development of methods to demonstrate the presence of estrogenic hormones in the blood and urine. The importance of this work lies not altogether in the additions made to our knowledge, but also in the recognition of a new method of approach in studying problems of physiology and pathology. It seems particularly appropriate, therefore, in a volume dedicated to him and to his achievements, to consider the present status of our knowledge in an associated field, namely, the detection of gonadotropic substances in the blood and urine.

The basis for these studies was laid in 1921 when Evans and Long[1] succeeded in producing very definite changes in the reproductive system of the white rat by the intraperitoneal injection of an alkaline preparation of bovine anterior hypophyseal substance. A few years later Smith,[2] and Smith and Engle[3] in this country, and Zondek and Aschheim[4] in Germany, observed that inoculations of fresh anterior pituitary gland tissue into immature mice and rats leads to a precocious sexual maturity. In 1928 Aschheim and Zondek[5] described their "pregnancy test" and showed that the urine of pregnant women contains large amounts of some hormone which has the property of inducing changes in the ovaries of laboratory animals. A standard procedure for the detection of gonadotropic hormones thus became available, and they were found in many body tissues and fluids under varying conditions. In 1929, Fels[6] and Fluhmann[7] noted their presence in the blood of pregnant women, Rössler[8] in the urine of women with chorionepithelioma, Fluhmann[9] in the blood of women following castration, in certain cases of amenorrhea and in the postclimacteric period, and Zondek[10] in 1932 described their appearance in the urine of men with teratomas of the testicle. Since then, many reports have appeared which have given us valuable information regarding the production of these substances, their properties and distribution, and many refinements in the technic of the test. In the Stanford Laboratory of Gynecology over 3,000 tests for gonadotropic hormones in blood, urine, and tissue extracts have been conducted between 1928 and 1937.

TYPES OF GONADOTROPIC HORMONES

With the announcement of Aschheim and Zondek that the injection of urine from pregnant women into immature rodents results in follicle growth and luteinization in the ovaries, it was at once assumed that it contained a principle derived from the anterior hypophysis. Although Engle, Orban and Watrin, and Collip early expressed doubt as to the validity of this conclusion, it was at once generally accepted and is still maintained by a number of authors. The work of the past few years, however, has brought forth a convincing array of facts which show that we are not dealing with a single anterior pituitary factor, but with a number of gonadotropic substances which may not have a common origin. I have summarized the most significant biologic differences between these hormones in a number of previous communications (Fluhmann[11-13]), and they indicate that such substances should be identified with one or the other of two important groups.

1. The "anterior pituitary sex hormones" are found in the blood and urine of normal women and in increased amounts in castrates, in some patients with amenorrhea, and in the postclimacteric period. Their prototype is found in various extracts prepared from anterior pituitary glands or in fresh hypophyseal tissue, and two noteworthy biologic reactions may be mentioned. In immature rats or mice they induce a rapid development of numerous follicles, some of which luteinize while others undergo atresia, so that in 96 or 120 hours, the ovary presents the picture of numerous small corpora lutea interspersed with atretic follicles. Second, they readily stimulate follicle growth, luteinization and hypertrophy of interstitial cells in the ovaries of hypophysectomized rodents. Following the work of Hisaw, Fevold, and their collaborators, and subsequent corroboration from Wallen-Lawrence, Evans and others, it is now believed that these changes are due to two distinct hormones which may exist in various proportions. In the first place there is anterior pituitary hormone-A, which stimulates the growth and maturation of graafian follicles. Second, there is anterior pituitary hormone-B, which causes a luteinization of granulosal and thecal cells.

2. The "chorionic gonadotropic hormone" is distinct from the first group in that it occurs in large amounts during pregnancy, in association with chorionepithelioma and hydatidiform mole, and in men with certain testicular tumors. It presents many biologic differences from the anterior pituitary hormones. For instance, in the immature rat ovary it induces follicle growth and luteinization, but the resultant histologic picture is characteristic. Instead of the large numbers of small, closely packed corpora lutea and atretic follicles which result from the administration of anterior lobe extracts, there are large corpora, normal developing follicles, and larger or smaller cysts lined with lutein cells. Moreover, these changes are believed to be due, not to the chorionic hormone alone, but to its action along with anterior pituitary factors already present in the normal animal. This is shown by the fact that in hypophysectomized rats the chorionic hormone fails to stimulate follicle development and only directly affects the ovarian interstitial cells. In spite of the repeated statement that the chorionic hormone is made up of two different elements (Novak[14]), no sound experimental evidence such as has been advanced for the anterior hypophyseal substance is as yet available.

The origin of the chorionic hormone is not known for a certainty. In many ways it seems to fulfill the requirements for a luteinizing factor from the anterior hypophysis, but on the other hand, there is good reason to believe that it may be produced by the placenta and by certain newgrowths. Until the final answer is obtained, it is very important that these two groups be kept distinct, and this is especially true in dealing with clinical problems. There are not only many biologic differences between the two types of hormones, but they also occur under very different physiologic and pathologic conditions.

ANTERIOR PITUITARY GONADOTROPIC HORMONES

The demonstration of this type of gonadotropic hormone depends on the employment of various modifications of the Aschheim-Zondek pregnancy test. Mice, rats, or rabbits may be used. The relatively small size of the mouse makes it a more sensitive test animal but a marked disadvantage is faced when extracts with toxic properties are injected. With any extensive studies of this nature it is desirable that a colony of mice be developed so that exact ages may be known and control observations made at different seasons to determine the time at which first estrus appears in the majority of animals. The work of Engle and Rosasco[15] and Hamburger[16] has amply shown the many errors which may arise from the common practice of purchasing mice from dealers and depending altogether on body weight as evidence of immaturity. In the Stanford Laboratory of Gynecology it has been found necessary to begin the test with mice eighteen to twenty days old, since sexual maturity occasionally may appear as early as twenty-five days of age.

The injections have been carried out in various ways, but two methods may be mentioned as standard procedures. First, the material to be tested may be given in single daily doses for three consecutive days and the animals sacrificed in 96 to 100 hours from the time of the first injection. This is generally done with extracts, but whenever blood serum or urine is tested directly, it is preferable to give a larger number of injections and to prolong the period of observation. For instance, in examining blood serum it has been our practice to inject 0.5 c.c. twice daily for four consecutive days and to autopsy the mice in 120 hours from the beginning of the experiment.

The end point of the test designating a positive result has received many interpretations. The stimulus of the gonadotropic hormone causes follicle development with or without luteinization in the ovary, and secondarily, enlargement of the uterus, establishment of the vaginal introitus, and estrous changes in the vaginal mucosa. It must

be remembered, however, that any changes affecting the uterus and vagina are secondary to ovarian changes, and therefore cannot be employed unless it has been very clearly demonstrated that the material being tested does not contain any estrogenic substances. As a case in point, Thomsen and Pedersen-Bjergaard[17] use the vaginal responses obtained in the mucification test for estrogenic substances (Fluhmann[18]) as criteria for stimulation of ovarian function in immature mice. It may no doubt prove a very delicate procedure for detecting gonadotropic hormones but is entirely dependent on the absolute absence of estrogenic hormone in the material originally administered to the animals. The final test for a gonadotropic hormone must depend on the clear demonstration of not only functional, but anatomic, changes in the ovary of the immature rodent. This is best shown by serial sections of ovaries fixed in Zenker's solution, mounted in paraffin, and stained by hematoxylin and eosin.

Two such effects are of significance in testing for the anterior pituitary gonadotropic hormone; (1) developing, or large atretic graafian follicles, and (2) corpora lutea, with or without imprisoned ova.* In the majority of instances, large follicles with or without signs of atresia in the ovary of the test mouse give evidence of anterior lobe gonadotropic hormone in the blood or urine. On the other hand, luteinization occurs also and is apparently dependent on a quantitative factor, although there is the possibility that it is due to a relative concentration of the "follicle-stimulating" and "luteinizing" hormones of the anterior lobe. In 71 positive tests with blood serum I found 15 instances of corpus luteum formation (Fluhmann[19]), and similar observations have been made by a number of other workers. Salmon and Frank[20] recently investigated this aspect of the problem, and rightly pointed out that either follicle-stimulating or luteinizing effects could be produced in immature (rat) ovaries by increasing or decreasing the dosage of given extracts, but it was not possible "to conclude whether one factor at different dosage produces both effects or whether two factors are present with different dosage thresholds."

The administration of untreated blood serum or urine from normal individuals to immature mice or rats fails to yield positive tests. The blood of 45 women with normal menstrual cycles regularly gave negative reactions (Fluhmann[19]), and Mazer and Goldstein[21] obtained positive results in only 2 out of 53 similar patients. This method is therefore of no use in studying the normal content of gonadotropic substances in blood or urine, although it is of distinct value in demonstrating increases of the hormone under various conditions. For this

*In addition, mention must be made of follicles with hemorrhage (APR-II of the original A-Z reactions) and lutein cysts, but these appear infrequently and are more characteristic of the chorionic hormone.

reason, a number of procedures have been developed to concentrate the amount of hormone present before the biologic test is conducted. Neumann and Peter,[22] Frank and Salmon,[23] and Freed[24] have described methods of extracting gonadotropic substances from blood, and similar procedures for urine have been reported by Zondek,[25] Katzman and Doisy,[26] Levin and Tyndale,[27] Katzman,[28] and others. Although the exact relationship between gonadotropic hormones in the circulating blood and those excreted in the urine has not been established by careful analysis, it is generally believed that they run parallel and are directly comparable.

There is, unfortunately, no uniformity of results in the studies conducted on the presence of gonadotropic hormones in the blood and urine of normal individuals. Soeken[29] reported positive tests in the urine of 24 out of 50 children, but his findings could not be corroborated by Schörcher[30] and Ehrhardt and Ruhl.[31] Katzman and Doisy[26] observed that between the ages of four years and puberty little or none of the gonadotropic substance was found, while at puberty an increased excretion occurred. The examination of the blood and urine of normal menstruating women also shows much contradiction. Frank and others[32] found that a maximal concentration of gonadotropic substances in the blood occurred from the sixth to the ninth days of the cycle, Frank and Salmon[23] from the ninth to the thirteenth days, while Neumann and Peter[33] noted an increase during the premenstruum. The studies conducted with urine are likewise confusing, but the available reports may be placed in two groups. In the first are those investigators who believe that the maximal excretion of gonadotropic hormone takes place during the premenstruum (Zondek[34] and Österreicher[35]). The second group (Katzman and Doisy,[26] Kurzrok and others[36]) finds the greatest concentration at about the middle of the menstrual cycle and feels that it represents an increased activity of the anterior lobe which is associated with ovulation. It is hoped that additional data on these important points will soon be available.

Although difficulty has been experienced in determining the occurrence of anterior pituitary gonadotropic hormone under normal conditions, it has been amply demonstrated that following a loss of ovarian function there is a marked increase both in the blood (Fluhmann,[7] Zondek,[37] Mazer and Hoffman[38]), and in the urine (Zondek,[39] Jeffcoate,[40] Mazer and Andrussier,[41] Brühl,[42] Österreicher,[43] Gostimirove,[44] and others). In my original studies,[7, 19, 45] an increase of gonadotropic hormone in the blood was found in 40 per cent of patients examined within three months after an operative castration and in 76 per cent after this period, in 60 per cent of women following an irradiation castration, in 60 per cent of postmenopausal women, and in a number of patients with long periods of amenorrhea. This

increase was observed as early as eight days following operative castration, and Österreicher[46] and Saethre[47] have demonstrated it in the urine of women as old as eighty and ninety years of age.

This observation is of considerable interest and two possibilities arise to explain the great increase of the hormone in the blood and urine. It may result from a nonutilization of the hormone by afunctional or absent gonads, but a number of associated findings support the view that it is due to an actual hyperfunction of the anterior hypophysis. (1) It is in keeping with the postcastration hypertrophy of the anterior lobe of women, which has been described by a number of authors (Tandler and Gross,[48] Kon,[49] Kolde,[50] Rössle[51]). (2) Anterior hypophyseal tissue from gonadectomized rats is much more potent to stimulate the ovaries of immature rodents than that from normal controls (Engle,[52] Evans and Simpson[53]). (3) Estrogenic hormones are inhibitors of anterior pituitary function so that this inhibition is removed with the cessation of ovarian function. (4) It has been possible to decrease the excessive elimination of gonadotropic hormone in menopausal patients by the administration of large dosages of estrogenic substances (Albright,[54] Frank and Salmon,[55] Zondek[56]).*

In the light of these findings the demonstration of excessive amounts of anterior lobe gonadotropic hormone in the blood and urine of women is interpreted as indicating a cessation of ovarian function and has been employed in a number of clinical problems. For instance, it is of value in the study of patients with amenorrhea, and positive tests are considered as evidence of a "primary ovarian failure" (Mazer and Goldstein[21]). It also has been used recently to determine the duration of gonadal function in women who have had hysterectomy with conservation of the gonads (Marx and others,[57] Tamis[58]).

It has been maintained that an increased elimination of anterior lobe gonadotropic hormone occurs in two important conditions in addition to lack of ovarian function. In the first place, Zondek[59] has reported its association with carcinoma of various pelvic organs in a large proportion of cases, and apparent corroboration has come from Winter[60] and Bandler.[61] However, these findings have not been substantiated by Frank[62] and Hamburger,[63] and in my own studies with blood no relation could be found between carcinomatous disease and increase of gonadotropic hormone in younger individuals. It must be

*However, the exact relationship between estrogenic hormones and overproduction of anterior lobe gonadotropic substances is not quite clear. It has been maintained that the latter occurs with the disappearance of the ovarian factors, but that is not necessarily true. A certain percentage of preclimacteric patients show an increase of gonadotropic hormones while still menstruating, and recent studies indicate that estrogenic substances are found in both castrates and postmenopausal women. The two groups of hormones may thus be present in large amounts at the same time.

pointed out, also, that the vast majority of reported positive results occurred in women in the preclimacteric or climacteric periods. Second, considerable discussion has centered on the presence of gonadotropic hormones in the urine of patients with acromegaly, hypophyseal tumors, prolonged increase of intracranial pressure or essential hypertension. In these instances there is likewise a diversity of opinion, positive tests having been reported by v. Morgitay-Becht and Miklos,[64] Kraus,[65] Hirsch-Hoffmann,[66] Kylin,[67] and McCullagh and Cuyler,[68] while Watts,[69] Fels,[70] and Scarf and Israel[71] have not been able to offer corroborative evidence. This aspect of the question should therefore remain sub judice for the present and await further studies.

CHORIONIC GONADOTROPIC HORMONE

The Aschheim-Zondek "pregnancy test" is the method employed for the detection of the chorionic gonadotropic hormone, and it has received widespread acceptance as a standard procedure. In this country the use of rabbits, as suggested by Friedman, has supplanted rats and mice as test animals, and the many writers who have reported on the subject attest to its great accuracy. The modified Friedman test employed in the Stanford Laboratory of Gynecology has given 99 per cent correct results in normal pregnancy, and our experience supports the recommendations recently made by Kelly and Woods.[72] The patients are instructed to limit fluid intake after 10:00 P.M. on the day previous to the examination, and the first specimen of urine voided the following morning is forwarded to the laboratory. The rabbits are adult females weighing no less than 3½ pounds, which have been isolated for a period of at least two days. They are anesthetized with ether, and a small incision is then made in the flank in order to inspect one of the ovaries. If small clear follicles are found, the ovary is dropped back into the abdominal cavity and the wound closed by interrupted stitches in the muscles and metal clips in the skin. An intravenous injection of 7 c.c. of the urine is made at once into one of the ear veins, and this is repeated a few hours later. The rabbits are examined forty-eight hours after the first injection, and a positive result is made from the presence of hemorrhagic or ruptured follicles in one or both ovaries.

Although the Friedman test is the method of choice for the routine examination of urine specimens, there is still a field of usefulness for the smaller rodents in making quantitative determinations of the hormone or for the study of blood or tissues. In contrast to the anterior lobe gonadotropic hormone, however, the rat is from 3 to 5 times more sensitive than the mouse to the chorionic substance (Hamburger,[63] Rowe and others,[73] Nelson and Overholser[74]). The same precautions should be employed in choosing suitable immature rats,

and we employ animals of from twenty-one to twenty-three days of age which have been bred in our own colony. Since the chorionic hormone usually occurs in very great amounts it is not necessary to concentrate either the urine or the blood, and, in fact, in doing quantitative tests it is usually necessary to dilute the original specimens. At least three rats should be used for each dosage level, and according to the original Aschheim-Zondek procedure 3 injections are given daily for two days and the animals sacrificed in 96 to 100 hours. Although some of the changes induced in the ovaries may be detected grossly, it is much better to study serial sections as in the case of the anterior pituitary gonadotropic substance. Three reactions have been described. "APR-I" is denoted by the presence of follicles, and these consist of normal developing follicles or small cysts lined with lutein cells. There is a striking absence of follicles showing chromatolytic degeneration, as seen with anterior lobe preparations. "APR-II" consists of hemorrhages into follicles or lutein cysts, and may be seen grossly as "Blutpunkte," but they do not occur as readily in rats as in mice. "APR-III" is indicated by corpora lutea, with or without imprisoned ova.

The chorionic hormone is not found in normal menstruating women, but occurs in very great amounts throughout the whole course of gestation in the human. It may be observed as early as during the first two weeks following conception, but it rapidly disappears within a few days after delivery. It is beyond the scope of this review to refer to the numerous studies on the amounts of hormone present at different stages of pregnancy, but attention must be directed to the recent careful work of Evans and his associates.[75] These authors emphasize two of their observations which have considerable clinical significance. In the first place, the amount of hormone in the urine maintains a high level throughout pregnancy, but there occurs an exceedingly steep hormone peak at a time which is quite accurately one month from the beginning of the first expected but missed menstruation. Second, they find hormone levels associated with normal gestation far greater than previously reported. In one case, over one million rat units were excreted during the course of twenty-four hours.

The wide variations of hormone levels encountered in different individuals, the high peaks observed by Evans, and the difficulties of accurate quantitative determinations render it very difficult to efficiently control any analysis of increase or decrease in the amounts of hormone present in pathologic conditions. It is of particular interest, however, that a number of investigators have reported excessive amounts of the chorionic substance in the blood and/or urine in hyperemesis gravidarum (Von Weymersch,[76] Anker and Laland,[77] Schoeneck,[78] Heim,[79] Ehrhardt,[80] Anselmino and Hoffmann[81]) and in the

toxemias of late pregnancy (Smith and Smith,[82] Heim,[79] Anselmino and Hoffmann[81]). The significance of this increase is difficult of interpretation, but is an observation worthy of further investigation.

The demonstration of the chorionic gonadotropic hormone in the blood and urine of women with hydatidiform mole and chorionepithelioma has been accomplished by many workers (Rössler,[8] Zondek,[83] Mazer,[84] Mack and Catherwood,[85] Kimbrough,[86] Mathieu and Palmer,[87] Zondek.[88] and others). It is a discovery of the utmost clinical significance and may be of great value from the standpoint of diagnosis and therapy. The occurrence of positive tests, and especially of tests increasing in intensity some weeks or months after a normal pregnancy or a hydatidiform mole, is very important in establishing a diagnosis of chorionepithelioma. It is also of inestimable significance in controlling therapeutic measures, since a persistence of the hormone in the urine may indicate an incomplete operation for a mole or the occurrence of metastases in chorionepithelioma. On the other hand, it must be recognized that there are some limitations to the usage of the test and the results must be clearly interpreted in the light of the patient's history. For instance, the chorionic hormone may persist in the blood and urine for as long as six weeks after the evacuation of a hydatidiform mole and therefore may not necessarily imply that an incomplete operation has been performed or that a chorionepithelioma is present.* It is also especially important to interpret with caution the results of quantitative determinations of the hormone. Zondek[88] states that the presence of 200,000 units or more of the luteinizing hormone in the urine favors the diagnosis of hydatidiform mole in the differentiation from a normal pregnancy. However, much higher values have been found in normal pregnancy; and again little or no hormone could be demonstrated in some instances of hydatidiform mole (Philipp[89]). In a patient with a chorionepithelioma uteri associated with extensive metastases to the lungs and liver I found smaller amounts of the chorionic hormone in the urine than are generally present during the course of a normal gestation.

The presence of a gonadotropic principle in the urine of men with teratomas of the testicle was first reported by Zondek[90] and has received abundant confirmation from Ferguson[91] and others. It also has been recently shown that this substance has the biologic properties of the chorionic and not of the anterior hypophyseal hormone (Fluhmann and Hoffmann,[92] Evans[93]). The test in such cases is of value in the differential diagnosis of testicular tumors and may be employed to determine the effectiveness of operative or irradiation therapy and the appearance of metastases following the eradication of the primary

*However, if the examination of the urine yields positive results after it has become negative, the finding is significant.

tumor.[1] In a recent communication, Hamburger[94] maintains that in addition to the group of newgrowths which produce the chorionic hormone, seminomas of the testis may also cause the appearance of a gonadotropic factor which has the characteristics of the anterior lobe follicle-stimulating hormone.

The development of methods to detect gonadotropic hormones in the blood and urine has contributed much to the progress of our knowledge. They have given us tests which not only are valuable for practical application but serve as guides in the investigation of obscure problems in physiology and pathology. In spite of the advances that have been made there is much to be accomplished in the future, and it is hoped that many more workers will adopt these procedures and contribute to this important field.

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THE INFLUENCE OF LACTATION ON THE IMPLANTATION OF THE MAMMALIAN EMBRYO

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THE attachment of the embryo to the uterine mucosa constitutes a critical period both in the development of the embryo itself and in the maternal processes of gestation. Prior to implantation the embryo has no organic connection with the maternal tissues and is indeed isolated from them by the more or less attenuated zona pellucida, which persists in some forms almost until this time. It is dependent for its nutrition partly on the reserve materials of the ovum and partly on the contents of the fallopian tube and uterus, which are absorbed in fluid form. Subsequently the embryonic and maternal tissues are in continuity and the nutrition of the embryo is then effected by the absorptive and phagocytic activity of the trophoblast and of the placenta. Implantation marks in the mother the transition from the preparatory postestrous reconstruction of the uterine mucosa and the phase of maximum secretory activity of the uterine glands to the formation of the definitive maternal placental tissues. Despite the importance of implantation in gestation and the considerable knowledge we possess of the morphologic changes which accompany it, we know relatively little of the physiologic processes concerned. Some light is thrown on these by the observations on the effect of lactation in retarding implantation which are reviewed in this paper.

Lataste recognized in 1882 that, in a number of mammals, the duration of gestation is prolonged during lactation. In his treatise entitled "Recherches de Zooéthique sur les Mammifères de l'ordre des Rongeurs." (1887) he stated: "Chez les Muridés, quand la femelle entre en lactation au début de sa grossesse, la durée de la gestation peut être de trois périodes génitales; dans tous les autres cas, elle n'est que de deux périodes." The "période génitale" of Lataste's Law is the approximately ten-day period of the pseudopregnant or mated estrous cycle of the mouse.

Although Lataste's most extensive researches on this problem were performed on the mouse, he showed that gestation is prolonged by lactation in *Dipodillus Simoni*, Lataste, and *Meriones longifrons*, Lataste, as well. Moreover he showed conclusively that the prolongation is due to an arrest in the development of the embryos at an early stage, before they have produced macroscopically distinguishable swellings of the uterus. He states that lactation continued for only three, five, or eight days from the time of parturition does not result in prolongation of gestation, but examination of his schedules reveals that while this conclusion was justified for three and five days' lactation, it was not in the case of eight days' lactation. Lataste also

showed that mammary activity below a certain intensity does not produce a retardation of development; thus the duration of gestation was normal in mice suckling only one or two young while it was prolonged in those suckling three or more young.

Daniel (1910) records ten instances of the prolongation of gestation from two to ten days in mice suckling from three to ten young. He concluded from these data that the period of gestation, in lactating mothers, varies directly with the number of young suckled. He found that the prolongation was approximately one day for each young suckled. Although his data are meager, they gain weight from the fact that only five females were used, three of which had two prolonged gestations and one had three. In each individual mouse, as well as in the series as a whole, the prolongation was greater, the greater the number of young suckled.

King (1913) showed that a similar prolongation of gestation during lactation occurs in the albino rat, *Rattus norvegicus albinus*, of the Wistar Institute strain. She records the duration of thirty-one pregnancies, varying from twenty-one to thirty-four days, in rats suckling from three to eleven young. In this strain the gestation period in nonsuckling females varies from twenty-one to twenty-three days. All females suckling six or more young had prolonged periods of gestation; of the nineteen rats suckling five or less young, gestation was not prolonged in the eight which subsequently gave birth to litters of five or less but was prolonged in the eleven which gave birth to litters of six or more. It is suggested that the suckling of a litter of six or more young lessens the food supply to the fetal young and so retards their development.

Kirkham (1916) investigated the stage of development attained by the embryos in a series of 21 mice suckling from three to eight young killed at daily intervals from six to twenty-four days *postpartum*. He compared these with a standard series of embryos from nonsuckling females which had become pregnant at the *postpartum* estrus. He found that copulation occurs within twenty-four hours of parturition and that in both suckling and nonsuckling animals the ova are in the 2-cell stage during the first and second days of gestation, are morulas during the third and fourth days and during the fifth day are blastocysts free in the uterine lumen. In nonsuckling females, in which the normal gestation period is twenty days, they become implanted at the close of the fifth day. Ten females suckling from three to eight young, when killed from six to fourteen days *postpartum*, all had blastocysts free in the uterine lumen. He concluded, therefore, that in the suckling mouse implantation occurs at the end of the thirteenth day of pregnancy (fourteenth day *postpartum*) and that this delay is due to the loss of surplus nourishment through the mammary glands, preventing the uterine mucosa from reacting to the embryos. The embryos in eleven suckling mice killed from fifteen to twenty-four days *postpartum* were implanted and exhibited retardation, compared with those of nonsuckling mice, of from three to fourteen days, except in the single instance where only 3 young were suckled in which there was no apparent retardation. He found no evident correlation between the amount of retardation and either the number of young suckled or the number of embryos in utero.

Kirkham, in a subsequent paper (1918), records the duration of pregnancy in nine mice suckling from one to four young. None of the four mice suckling one or two young showed any prolongation, four mice suckling three or four young showed prolongations of from nine to eleven days and one mouse suckling three showed none. A series of twenty-two mice, which were each suckling four young and were killed at intervals of from two to twenty-five days *postcoitum*, were examined, and the contained embryos were compared with those from nonsuckling females. None of those killed up to the thirteenth day had implanted embryos, whereas eleven of the twelve killed subsequently had. The eleven animals with implanted embryos exhibited

retardations varying from two to twelve days. In another experiment involving sixteen mice, the animals were allowed to suckle the whole litter born at first but subsequently all but one were removed at intervals, varying from one to thirteen days *postpartum*. All were killed on the thirteenth day *postpartum* and the embryos examined. All the six that suckled the whole litter for more than six days, that is after the normal time of implantation, showed retardation of from five to eight days. Some of those that suckled the whole litter for six days or less exhibited retardation of up to five days while others showed none.

Mirskaia and Crew (1931) record the duration of pregnancy in 19 mice, suckling from 3 to 8 young, of which 7 were primiparas, having become pregnant at their first *postpartum* estrus, and 12 were multiparas. They found that in all cases gestation was prolonged, the period varying from six to sixteen days without reference either to the number of young in the uterus or suckling. They found no support for Kirkham's view that the functioning mammary glands exert an inhibitory action on the uterus, but they suggest that the delayed implantation is due to inability of the corpus luteum to provide sufficient luteal hormone for implantation and lactation to take place simultaneously.

Hain (1934) records variations between twenty-two and thirty-six days in the duration of pregnancy in fifteen albino rats suckling from 3 to 11 young. She concluded that, unlike the mouse, the duration of pregnancy in the rat may not be prolonged when only 3 or 4 young are suckled. It is always prolonged when more than four young are suckled. She found no correlation between the number of young suckled and the duration of the prolonged gestation. Hain found no evidence that the number of embryos in the uteri affects the duration of gestation when five or less young are suckled, as was suggested by King (1913).

Lataste's pioneer work seems to have been overlooked by all the subsequent workers referred to above, since in none of the papers quoted is there any reference to it.

The author (1935), when investigating the estrous cycle of the common shrew (*Sorex araneus*, Linnaeus) with material trapped in the wild state, found among the pregnant animals an unexpectedly high proportion with blastocysts free in the uterine lumen. In this species, it is possible to distinguish females that are pregnant for the first time from those that are parous, and further analysis revealed that this unexpected preponderance of the unimplanted uterine blastocyst stages was almost entirely confined to the group of parous females. Moreover in the common shrew the majority of females become pregnant at the *postpartum* estrus, lactating and gestating simultaneously. The distribution of the ninety-nine pregnant animals obtained, according to the stage of the contained embryos, between first and subsequent pregnancies, is summarized in Table I.

TABLE I

STAGE OF EMBRYOS	FIRST PREGNANCIES	SUBSEQUENT PREGNANCIES
Fertilized ova in the fallopian tubes	7	11
Blastocysts free in the uterine lumen	3	26
Implanted embryos	32	20
Total	42	57

The relative durations of these three stages in nonsuckling shrews are not known, but it may be assumed for the present purpose that they do not differ widely from those in the mouse which Kirkham (1916) has shown to be approximately four, one, and fifteen days, respectively. We would expect, on this assumption, to find in a random sample of pregnant animals 20 per cent with fertilized tubal ova, 5 per cent with free uterine blastocysts, and 75 per cent with implanted embryos. The fact that in many species of wild mammals the pregnant females approaching full term are more difficult to trap, presumably because they are less active, provides a satisfactory explanation of the relatively small number with implanted embryos actually obtained. The relative numbers obtained of the two earlier stages cannot be accounted for in this way and are much more significant. The very much greater relative frequency of free uterine blastocyst stages in parous animals can be satisfactorily explained only by postulating an arrest in the development of the blastocysts and a delay in implantation during lactation such as occurs in the small rodents.

A similar prolongation of gestation during lactation probably occurs in the lesser shrew (*Sorex minutus,* Linnaeus) also, for among 47 pregnant animals, 4 had tubal ova, 12 had free uterine blastocysts, and 31 had implanted embryos (Brambell and Hall, 1937).

The bank vole (*Clethrionomys glareolus britannicus,* Miller) also exhibits a preponderance of free uterine blastocyst stages among wild pregnant females (Brambell and Rowlands, 1936). In this species it is not possible to distinguish parous animals from those pregnant for the first time, except in special instances. However, animals taken during the early part of the breeding season (in April and the first half of May) cannot have been suckling, whereas the majority of those taken later in the season were in lactation. Comparison of the number of tubal and unimplanted uterine stages in these two groups, as shown in Table II, reveals a very marked preponderance in the proportion of free uterine blastocyst stages in the latter part of the season.

TABLE II

STAGE OF EMBRYOS	BEGINNING OF SEASON TO MAY 15	MAY 16 TO END OF SEASON
Fertilized ova in the fallopian tubes	23	26
Blastocysts free in the uterine lumen	8	42
Total	31	68

This species appears much more difficult to trap in the later stages of pregnancy than the common shrew since of 179 pregnant animals obtained only 80 had implanted embryos. This does not affect the

present issue, since it is the relative preponderance of free uterine blastocyst stages as compared with tubal stages in the group including all suckling females which indicates delayed implantation during lactation. There is thus very strong evidence that such delay occurs in this species.

Information regarding the growth of the corpus luteum during pregnancy in this species was obtained by measuring the diameters of the corpora lutea of pregnancy of all the animals available and calculating the mean diameter of those of each. By arranging the pregnant animals in order according to the stage of development of the contained embryos, from the earliest to the latest, and plotting the mean diameters of the corpora lutea against the position of the corresponding animal in this series it was possible to arrive at a graphical represen-

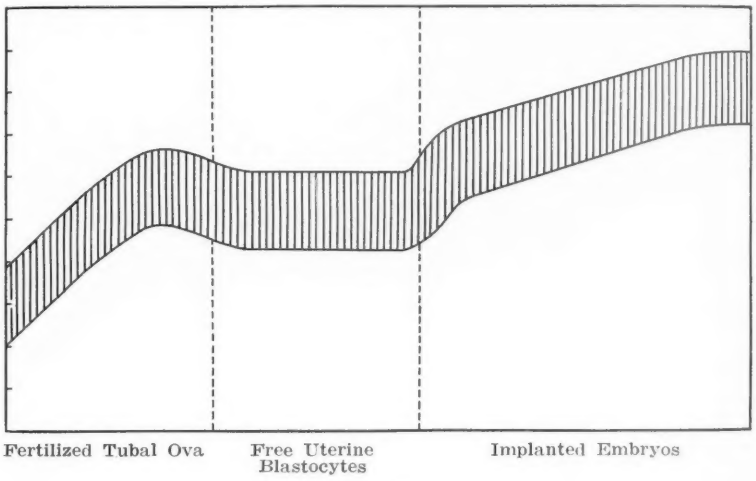

Fig. 1.

tation of the growth curve of the corpora lutea during pregnancy. This curve is represented diagrammatically in Fig. 1. It is apparent that the corpora lutea grow rapidly from the time of ovulation until the tubal ova are in the 8-cell stage. Thereafter there is no growth and there is even an indication of a slight decrease in size, until implantation occurs, when a second but less rapid growth phase begins and continues without interruption until parturition.

Assuming that, as in the mouse, without delayed implantation due to lactation, free uterine blastocyst stages should be one-fourth as numerous as tubal stages, and since there are 49 tubal stages, then the excess over twelve or approximately 75 per cent of the free uterine blastocyst stages must be assumed to be in a state of arrested development and delayed implantation due to lactation. The data on which Fig. 1 is based therefore clearly indicate that, when the development of the blastocysts is arrested and their sojourn free in

the uterine lumen is prolonged by lactation, the growth of the corpora lutea is arrested also. So far as we are aware these data provide the only information available regarding the growth of the corpora lutea during gestation prolonged by lactation.

The researches summarized above amply confirm Lataste's original statement that gestation may be prolonged during lactation in the small rodents and show that a similar prolongation occurs in the shrews also. It is only in the rat and the mouse that sufficient records are available to warrant any conclusions being drawn regarding the relation, if any, between the number of young suckled or the number of embryos in the uterus and the duration of the prolonged gestation. Examination of these records provides no evidence that the number of embryos in the uterus has any influence on the duration of gestation. Moreover a priori such influence seems improbable since the prolongation is due to delayed implantation and would therefore have to be exerted by the embryos while still in the blastocyst stage free in the uterine lumen. This suggestion need not be considered further unless evidence is forthcoming in support of it. The possibility of a relation between the number of young suckled and the duration of gestation cannot be disposed of so easily. Lataste observed that gestation is not prolonged in the mouse unless more than two young are suckled, and that in the rat it was not prolonged when three young were suckled. He suggested that the minimum number of young necessary to cause prolongation may vary in different species. Subsequent researches confirm this conclusion so far as the rat and mouse are concerned. Daniel's data, although too meager to be conclusive, suggest that the duration of gestation may be directly proportional to the number of young suckled in the mouse; a conclusion which he attempted to formulate as a general law. Although all subsequent workers failed to confirm Daniel's conclusions on the basis of their individual results, examination of all the records available, compiled from several sources, seems desirable before arriving at a definite conclusion. The data given in Table III show the prolongations in days of 67 pregnancies in mice suckling litters of known size. These have been extracted from the papers referred to by Lataste (1887), Daniel (1910), Kirkham (1916 and 1918), and Mirskaia and Crew (1931). Only records of completed pregnancies or of animals killed after the embryos had become implanted, when the delay in implantation could be estimated accurately by comparison with embryos of nonsuckling females, have been employed. In calculating the prolongation twenty days has been taken as the normal gestation period, except in the case of the records of Mirskaia and Crew who employed a strain in which nineteen days was the normal.

The maximum of twenty-two days, observed in two instances only in animals suckling one or two young, cannot be regarded as a significant prolongation of the normal gestation period, since this is known to exhibit variations of one or two days, especially in animals

TABLE III

PROLONGATION IN DAYS	NUMBER OF YOUNG SUCKLED									
	1	2	3	4	5	6	7	8	9	10
16								1		
15							1			
14							1			
13				1	1	4				
12			1	3		3	1			
11				2			1	1		
10			1	2		1	1	1		2
9			1	1				1		
8				2	1	1		1		
7				1		1	1	2		
6				2						
5			1	1	3	1				
4				3		2				
3					1					
2	1	1	1	1						
1			1							
0	2	2	2							

not belonging to a single inbred strain. Animals suckling three young may or may not have a prolonged gestation, but all those suckling more than three exhibit prolongations of from two to sixteen days. Statistical examination of these data for animals suckling three or more young shows that a straight regression line of the form:

$$y = 0{\cdot}84 + 3{\cdot}94$$

can be fitted (where y = the prolongation in days and x = the number of young suckled). Testing by means of the table of t (Fisher, 1930) with $t = 3{\cdot}2$ and $n = 59$ the value of P is found to be below 0·01 and the regression must therefore be regarded as significant. Although a direct relation therefore exists between the number of young suckled above three and the duration of gestation, it does not approximate to the one day for each young suckled, as stated by Daniel (1910), and the individual variation exhibited is very wide.

Similar data for the albino rat, compiled from the records of Lataste (1887), King (1913), and Hain (1934), are given in Table IV. Since in this species the normal gestation period varies from twenty-one to twenty-three days, the prolongation was calculated by subtracting 22, the mean gestation period of nonsuckling females. Therefore, in Table IV, prolongations of one day cannot be considered significant.

It is apparent that the gestation period in the rat may or may not be prolonged when three, four, or five young are suckled but that it is invariably prolonged when more than five young are suckled. The rat therefore differs from the mouse in the minimum number of young necessary to ensure prolongation. Moreover it can be seen from a glance at Table IV that a correlation exists between the number of young suckled and the duration of gestation. Probably the fact that almost all the data are derived from animals belonging to the inbred

TABLE IV

PROLONGATION IN DAYS	NUMBER OF YOUNG SUCKLED								
	3	4	5	6	7	8	9	10	11
14								1	
13							1		1
12							1		1
11									1
10									
9							1		
8						1		1	
7			1		1	1			
6		1			1	1			
5	1	1		1		1		1	
4	1			3					
3		2	1		2				
2	1	1	1						
1	2	2	3						
0	4	2	2						

Wistar Institute strain of rats accounts for the greater uniformity of the results as compared with the data on the mice, which were drawn from animals derived from many different sources. The linear regression line derived from Table IV is of the form:

$$y = 1{\cdot}33x - 3{\cdot}43.$$

It is clearly established that when lactation and gestation occur simultaneously, following the *postpartum* estrus, the duration of gestation is prolonged, provided that the number of young suckled exceeds a minimum which is characteristic for each species. The prolongation tends to be directly proportional to the number of young suckled but the individual variation is wide. This prolongation is due to delay in the implantation of the blastocysts in the uterine mucosa and an arrest in their development and is accompanied, in at least one species (bank vole), by an arrest in the development of the corpora lutea. When lactation is terminated by the removal of the young during the phase of arrest implantation follows rapidly.

The elucidation of the mechanism by which lactation brings about a temporary inhibition of implantation presents a difficult problem. Yet many lines of evidence converge upon it and it may well be that

we possess all the information necessary for its solution. It is obvious that the inhibition must be exerted, directly or indirectly, on the uterine endometrium or on the blastocysts or on both simultaneously.

The postestrous changes in the pregnant or pseudopregnant animal result in a progestational condition of the uterine endometrium which is invariably accompanied by sensitivity to mechanical stimuli. This was first experimentally demonstrated by Loeb (1908), who found that operative injury of the sensitive endometrium of the guinea pig resulted in the production at the site of injury of placentomas which resembled histologically the maternal placental tissues. Later Frank (1911) ascertained that a similar sensitivity exists in the rat and that in this animal placentomas could be produced experimentally during lactation, a discovery which was subsequently corroborated by Corner and Warren (1919) and by Long and Evans (1922). Although in the pseudopregnant rat, the sensitivity is at a maximum about the fourth day after estrus and has disappeared before the onset of the next estrus, the last named authors showed that it persists during lactation from the fourth to the sixteenth day *postpartum*. A similar sensitivity of the uterine endometrium in the lactating mouse was demonstrated by Parkes (1929).

It is well known that the sensitization of the endometrium and the other progestational changes in the uterus are effected by the action of the luteal hormone progesterone. Complete sensitization of the uterus can be produced experimentally by injection of this hormone, provided that, if the animals are immature or have been ovariotomized a considerable time previously, an initial sensitizing dose of estrin is administered some time before the progesterone.

Another function of the corpus luteum, which may or may not be independent of the progestational effect, is that of inhibition of estrus during either pregnancy or lactation. Estrus does not recur, after the *postpartum* estrus, during lactation in either the rat or the mouse, provided that a litter of normal size is being suckled. Estrus reappears in the suckling mouse about twenty-five days after the *postpartum* estrus (Parkes, 1926a) and in the suckling rat from twenty-five to forty days (Long and Evans, 1922). Mice suckling less than three young exhibit no such postponement of estrus, which then recurs at normal intervals during lactation (Parkes, 1926a). Estrous symptoms can be experimentally induced during lactation in mice suckling three or more young by the injection of the hormone estrin but the minimum amount of hormone necessary increases directly with the number of young suckled (Parkes and Bellerby, 1927). This phenomenon of inhibition of estrus during lactation in the mouse, appearing only when more than two young are suckled and exhibiting an intensity proportional to the number suckled has a curious, perhaps significant, resemblance to the phenomenon of prolonged gestation during lacta-

tion. Parkes and Bellerby (1927) showed further that the inhibitory effect of lactation on estrus is exerted via the ovaries, and hence presumably by the corpora lutea, for mice ovariotomized early in lactation required only small amounts of estrin to induce estrous symptoms in them, irrespective of the size of litter suckled.

These considerations lead to the conclusion that the hormone progesterone, secreted by the corpora lutea, induces the progestational changes in the uterus and an appropriately sensitive condition of the endometrium for implantation to take place during lactation. Moreover the activity of the corpora lutea, so far as inhibition of estrus is concerned, actually increases with the number of young suckled. Since the ability of the endometrium to react to suitable stimuli by the production of maternal placental tissues during lactation in both the rat and the mouse must be admitted, the theory that delayed implantation is due to inactivation of the uterus is clearly invalidated, whether caused by loss of surplus nourishment through the mammary glands, as suggested by Kirkham (1916), or by insufficiency of luteal hormone for implantation and lactation to take place simultaneously, as suggested by Mirskaia and Crew (1931), or by any other means. Indeed Mirskaia's and Crew's theory must, in any case, be discarded since, as has been pointed out by Hain (1934), lactation can continue after the ovaries have been removed experimentally.

Since the corpora lutea are able to perform both the progestational and estrus-inhibitory functions in nonpregnant lactating rats and mice, it follows that the second growth phase in their development during pregnancy, which has been observed in mice (Deanesly, 1930) and which is delayed until implantation occurs in lactating bank voles, is not essential for the performance of these functions. It is probable that this second growth phase is stimulated by the process of implantation and that it is in the nature of a preparation for the luteal activity during the placental phase of gestation, since it occurs only during pregnancy.

The alternative theory that the delayed implantation is brought about by inhibition of the blastocysts remains. It is, at first sight, more difficult to conceive how this could be effected, since the blastocysts are free in the uterine lumen and have no organic connection with the parent. Yet, by a process of elimination, it appears the more probable explanation. The occurrence of delay in implantation during lactation both in small rodents and in shrews is significant in this connection, for the method of implantation differs widely in the two cases. Implantation in the mouse and its allies is of the excentric type, in which the blastocyst, while still very small, becomes lodged in a pit or depression in the endometrium which closes over it and shuts it off from the rest of the uterine lumen. Consequently the blastocyst undergoes comparatively little further development after

passing from the fallopian tube into the uterus before implantation occurs. Implantation in the shrews, on the other hand, is of the central type in which the blastocyst, after reaching the uterus, enlarges considerably and, by distending the uterine lumen, becomes lodged in a spherical chamber. This chamber is of much greater diameter than the rest of the uterine lumen and opens freely into it. It is only after the formation of this chamber that the embryonic trophoblast becomes attached to the uterine epithelium with which it is in contact. Thus, while the blastocyst of the mouse undergoes little further development after reaching the uterus before implantation occurs, that of the shrew increases in size from 70 to 100 μ in diameter up to 800 to 1,000 μ before the zona pellucida disappears and the trophoblast becomes attached to the endometrium. Since the unimplanted blastocysts in the majority of parous shrews recorded above were of small or intermediate size, the arrest in their development during lactation must have taken place before they had attained the necessary size to become implanted. This suggests a positive inhibition of the blastocysts themselves, as distinct from an arrest in development occasioned by a failure on the part of the endometrium to react at the appropriate time and so provide the environment necessary for their further development.

The only suggestion that has been advanced as to how lactation could affect the embryos is that it lessens their food supply and so retards their development (King, 1913), but this was advanced before Kirkham had demonstrated that the arrest was prior to implantation. The intensity of mammary activity, in forms in which the milk secreted cannot be measured directly, can be estimated from the weight increments of the young during the period when they are entirely dependent on the mother for their nutrition. Parkes (1926b) has provided data of the average daily increments of young mice according to the size of litter suckled which admit of such an estimate. These data show that the daily weight increments rise at first to a peak between the fourth and eighth days *postpartum*, attained on an average for all litters on the seventh day, and thereafter fall off until

TABLE V

SIZE OF LITTER	NUMBER OF LITTERS OBSERVED	AVERAGE TOTAL INCREMENT DURING FIRST 15 DAYS GRAMS
1	1	12·2
2	2	14·5
3	5	14·82
4	7	19·08
5	9	19·7
6	8	21·42
7	15	24·57
8	12	25·52
9	5	25·56
10	2	24·5

the young begin to eat solid food about the fifteenth day. Thus mammary activity reaches a maximum about the time when the embryos, of a pregnancy originating at the *postpartum* estrus, would be implanting if their development were not arrested. Further, as might be expected, the intensity of mammary activity is proportional to the size of litter up to seven or eight but shows no further increase with larger litters. This is shown by the data of the average total increments of litters of 1 to 10 during the first fifteen days given in Table V.

Thus, if the drain on the maternal organism through milk secretion were the cause of prolonged gestation, the fact that it is at a maximum at the time when implantation would otherwise occur and decreases thereafter and is proportional to the number of young normally suckled, would account for many of the phenomena observed. Yet that it could inhibit the development of the blastocysts prior to implantation seems impossible, since, owing to their small size, their nutritional requirements could impose only an infinitesimal drain upon the parent organism. Moreover prolonged gestation occurs when only 3 to 6 young are suckled, yet the maximum activity of the mammary glands is not attained when less than seven young are suckled.

We are, therefore, forced to the conclusion that the explanation of the problem of how lactation brings about prolonged gestation is yet to be found. The evidence available appears to us to point to an inhibitory effect on the blastocysts themselves, exerted otherwise than through lack of nutriment, rather than to an effect on the uterine endometrium. Moreover, since the blastocysts are at this time free in the uterine lumen, without any organic connection with the parent, it would appear that this inhibition must be exerted through the medium of some substance secreted into the uterine lumen. It may be that this is a growth-stimulating substance, like that known to occur in certain embryonic tissue extracts, but of this there is at present no direct evidence.

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IDENTIFICATION AND SIGNIFICANCE OF SPIROCHETES IN THE PLACENTA

A Report of 105 Cases With Positive Findings

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THE difficulty in the diagnosis of syphilis in pregnancy is not fully appreciated by the general practitioner. There is no entirely satisfactory test. It is exceptional for the blood of a newborn syphilitic baby to give a positive serologic reaction; and most syphilitic babies born at full term show no gross syphilitic lesions. The placenta of the full-term syphilitic baby is usually normal in appearance; the classical description in textbooks is rarely found, and when found is generally due to prematurity.

Two facts stand out prominently in the diagnosis of syphilis in pregnant women. First, in any woman syphilitic infection commonly occurs with no signs of primary chancre, rash or mucous patches, and second, the supervention of pregnancy seems to attenuate this infection.

Stokes[1] refers to the "suppressing effect of pregnancy" as placing syphilis in women in a field by itself, and states that the infection is to some extent inhibited by the pregnancy. Moore[2] says: "If infection occurs early in pregnancy, the usual early manifestations of syphilis are much milder than if she is infected independently of pregnancy. Many fail to develop any of the usual early lesions of syphilis." Halbrecht[3] states: "We cannot rely on the Wassermann test in cases of hereditary syphilis, and it is most probable that fetal malformations represent forms of hereditary syphilis in the second and third generation." An illustration of the attenuation of syphilis is seen in the infrequency of complications of the nervous system following such cases. Zabriski[5] states that women who undergo several pregnancies are less liable to neurosyphilitic lesions than primiparas or sterile women. He suggests that there may be some immunizing power in the cholesteremia that accompanies pregnancy. Others have attributed the diminished virulence to the effect of iodine from the hyperactive thyroid. Brown, quoted by Zabriski,[6] has shown that extirpation of the thyroid in rabbits has a definite effect on the rapidity of development of primary and secondary lesions of syphilis. Routh[7] suggests an antispirochetal action of "placental ferments" or "syncytial toxins" that break up the spirals into granules and diminish their virulence.

As a result of attenuation—or some other factor—the Wassermann reaction is commonly negative in the syphilitic woman who is pregnant.

Guéniot[8] reported before the French Academie de Medecine in 1934, 1,119 cases of pregnancy where the clinical histories were such as to justify a suspicion of syphilis. In only 3.75 per cent was the Wassermann positive. "It seems," he states, "as though there were in pregnancy a factor which attenuates and some-

times renders negative the Bordet-Wassermann reaction.'' Other French authors have reported similar low percentages: Nobécourt[9] reported 18 per cent; Brindeau 9.6 per cent. Halbrecht[4] points out that antisyphilitic treatment may result in a negative Wassermann reaction even though the spirochetes are still present in the system. For this reason antisyphilitic treatment should always be resumed during the course of every pregnancy where there has been a syphilitic history. In fact, it is doubtful if the system, once infected, is ever completely cleared of spirochetes. Warthin[10] says: ''I have never seen at necropsy a case of perfectly healed syphilis . . . there is no evidence pathologically that the case of syphilis ever becomes wholly free from spirochetes.''

While it may be admitted that during the course of pregnancy the determination of syphilis is difficult, there is yet a prevailing belief that the birth of a healthy baby is a good indication that the child is free from syphilis. This is not true. The baby may look healthy; the placenta normal to gross appearance; the cord Wassermann negative; and yet the child may be afflicted with congenital syphilis. It is also a common belief that the placenta will tell the tale, if not to the naked eye, at least under the microscope. But the ground for this belief is now called in question.

Montgomery[11] says: ''Those histologic changes which we have attributed to syphilis are due to nothing more than the immaturity of the placenta and the accumulation of edema.'' McCord[13] commenting on Montgomery's article says: ''Formerly I believed that there was a definite histologic appearance of the placenta that was a constant pattern for syphilis, but I no longer think that is true.'' Ingraham, Jr., and Kahler,[14] in a masterly review of the literature on the subject, conclude: ''The diagnosis of syphilis in the mother or her child after birth is no easy matter, if you would approximate a hundred per cent accuracy . . . nothing short of a demonstration of treponemas in the fetal circulation of the placenta is indicative of a positive diagnosis of syphilis in any one but the mother.''

Since in the presence of syphilis characteristic lesions are not to be expected in the mother, in the child or in the placenta, and since the serum reaction in such cases for both mother and child is commonly negative, the identification of spirochetes that may be present in the placenta becomes a matter of prime importance.

The introduction in 1906 of the Levaditi method of silver impregnation in the exploration of tissue for spirochetes has been of the utmost service. But in the examination of the placenta the results have been disappointing. The long and tedious process involved in the search for the spirochetes in the placenta has been rewarded only too frequently by negative findings in cases where the clinical symptoms clearly indicated the presence of syphilis. Montgomery[12] speaking of the *Treponema pallidum* says: ''Their discovery in the placental substance happens so infrequently as to be of negligible value pathologically.'' A possible reason for this infrequency lies in the fact that the spirochetes are not equally distributed throughout the placenta, but are commonly clustered together in small foci, with large areas of the placenta totally free from their presence. Thus an indiscriminating

search in the placenta may readily fail to reveal spirochetes which are actually present. That eggs are not found lying about the barnyard is no proof that they are absent from the nests in the henhouse.

It has seemed to us that a more systematic method or search in the probable localities might give a higher percentage of positive findings. Boyd[15] states: "In congenital syphilis the primary lesion is in the placenta. There is, therefore, no primary stage in the child." That is to say, the primary focus being located in the placenta, it is not to be expected that there will be a widely disseminated lesion, but rather that there will be one or more sharply localized foci.

In the Obstetrical Department of the American University of Beirut, we have not felt justified with burdening our laboratories with routine Levaditi impregnation for all delivery cases in the hospital. We have contented ourselves with such examinations only in special cases. In cases of suspicious history, of positive serum reaction, of stillbirths, of prematurity, of placenta previa, of abortions, or of cesarean section, the examination has been made routine. In other cases where there is no suspicion of syphilis, the placentas are labelled and stored in a large jar of 5 per cent formalin until the baby is discharged from the hospital. In cases of neonatal death, or of any abnormality or lesion in the baby, the placenta is sent to the laboratory for examination by the Levaditi method.

In this way 145 placentas were chosen for examination from a total of 667 deliveries in the hospital during the two years, 1934 and 1935. In these 145 placentas, spirochetes were demonstrated in 105 cases, an apparent incidence of syphilis in the total number of pregnancies of 15.7 per cent. Among these 105 cases of positive findings were those who came to the hospital because of complications which had resulted from the presence of syphilis, such as abortions, stillbirths, polyhydramnios, and premature labor. For this reason the incidence of syphilis in the hospital is greater than it would be in cases of pregnancy delivered outside the hospital.

The modification of the silver impregnation method of search for spirochetes in the placenta consists in a more discriminating choice of sections and a more systematically directed hunt in the sections for the nests of spirochetes.

THE TECHNIC

The fresh placenta is placed in a flat jar containing 10 per cent neutral solution of formalin for twenty-four hours. Then it is cut through its longest diameter and a ribbon half a centimeter in thickness is removed. This is cut into pieces 3 or 4 cm. in length and allowed to fix in a 10 per cent neutral solution of formalin for three weeks or more, the longer the better. Twin blocks from each of these pieces are cut, one for ordinary paraffin inclusion, and the other, for silver impregnation. Sections from each of the paraffin blocks are stained with eosin and hematoxylin.

The silver impregnation method used is Nyka's modification of Levaditi's silver technic:[16]

After fixation for at least three weeks, proceed as follows:

1. Wash in 96 per cent ethyl alcohol for twenty-four hours.
2. Wash repeatedly in distilled water for twenty-four hours, changing the water often.
3. Impregnate for two to four days in 1.75 per cent silver nitrate solution in bidistilled water at 37° C.
4. Wash rapidly in bidistilled water.
5. Reduce for twenty-four hours in the following solution:
   Pyrogallic acid 4 per cent 90 c.c.
   Pyridine 17 c.c.
   Acetone drop by drop to dissolve the precipitate.
6. Wash rapidly in bidistilled water.
7. Dehydrate in alcohol.
8. Clear in xylol.
9. Include in paraffin.
10. Cut sections, mount, and after clearing in xylol, mount in Canada balsam.

In the eosin and hematoxylin sections the Warthin criterion is looked for, i.e., blood vessels showing mild peri -and endarteritis, with plasma cells and lymphocytes in the form of mild perivascular infiltration. The caliber of the blood vessel is strikingly narrower than normal on account of this inflammatory process (Figs. 1, 3, and 4). Spirochetes cannot be identified by the eosin and hematoxylin stain, but the presence of the Warthin criterion suggests the likelihood of finding the spirochetes in the Levaditi twin section. Infarction, hyalinization, deposition of lime salts, or ordinary thickening of the blood vessels (Fig. 2) does not indicate or suggest the presence of spirochetes.

In the silver impregnated twin sections, foci are looked for under the low power which take a pale yellow stain and are surrounded by a dark powdery deposit (Fig. 5). In these and especially around them the spirochetes are usually found (Fig. 6). These foci are generally located near the amniotic surface of the placenta, but may be found in other sites. They have no direct relation to the vascular lesions already described in the eosin and hematoxylin sections but may be found in their vicinity. Their appearance and topography convey to the careful observer the impression of miliary gummas. We have not been able to find spirochetes in the placental infarcts.

Under the oil immersion the spirochetes were numerous and easy to find in some cases (Figs. 6 and 7), while in others they were very few and surrounded and covered by a blackish brown precipitate, possibly the remnants of broken spirochetes.[17] It is, therefore, necessary to examine sections from all the blocks prepared before pronouncing a placenta free. Four out of five blocks examined might be negative while a fifth might show spirochetes in abundance.

While we have no proof as to the exact nature of these spirochetes, the forms seen in the Levaditi silver sections cannot be differentiated morphologically from the *Treponema pallidum* as to length, thickness, number of coils and general appearance. The large percentage of agreement between the clinical evidence of syphilis and the laboratory findings of spirochetes in the placenta, as we shall see later, is a strong indication that they are, at least for the most part, the *Treponema pallidum* of syphilis. These findings have been corroborated by E. W.

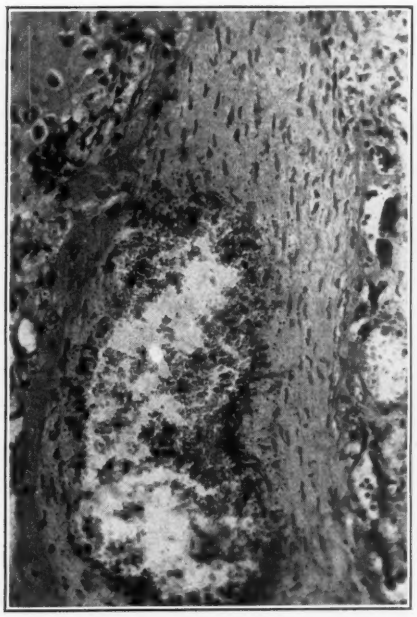

Fig. 1.

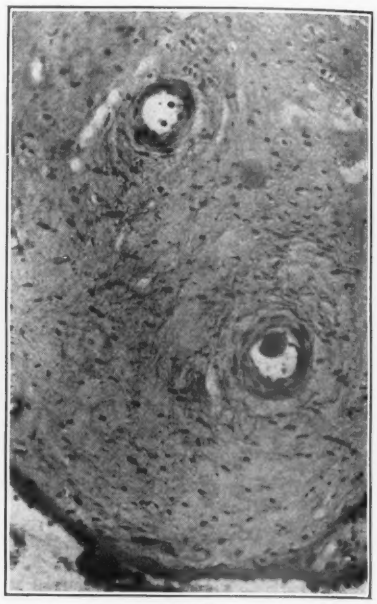

Fig. 2.

Fig. 1.—Normal blood vessel of the placenta. (×200 mag.)

Fig. 2.—Normal thickening of blood vessel often found in full-term placenta. (×200 mag.)

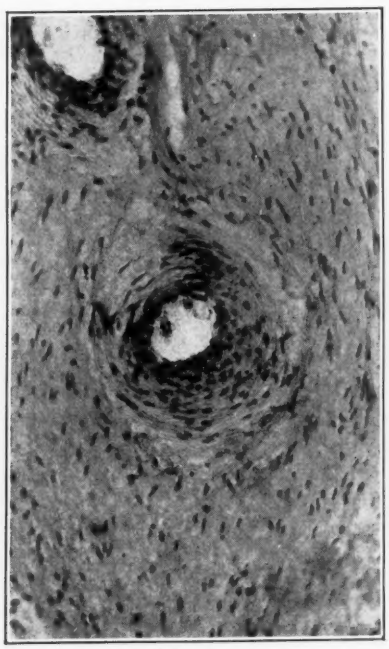

Fig. 3.

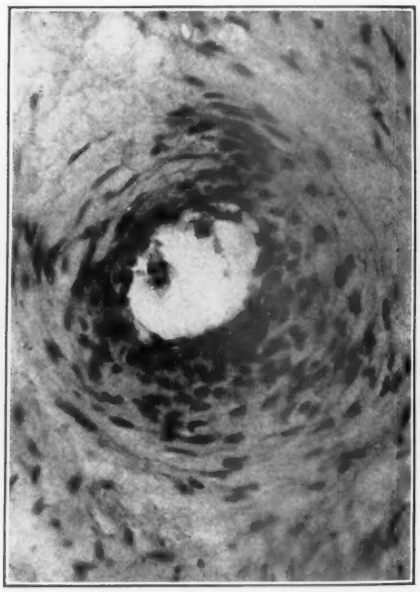

Fig. 4.

Fig. 3.—Case 86. Table VI. Warthin's criterion.—Blood vessel showing peri- and endarteritis with lymphocytic and plasma cell infiltration. (×200 mag.)

Fig. 4.—Same case as Fig. 3. (×400 mag.)

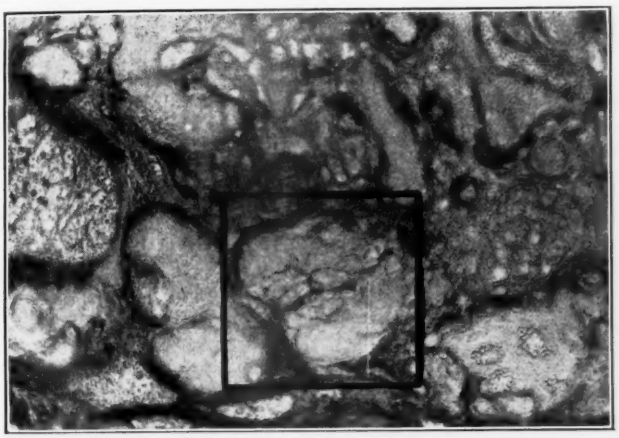

Fig. 5.—Case 86. Levaditi silver impregnation. Square indicates the gummatoid lesion in which the spirochetes are found. (×200 mag.)

Fig. 6.—Same as Fig. 5. Oil immersion showing spirochetes and dark brownish black granules. (×900 mag.)

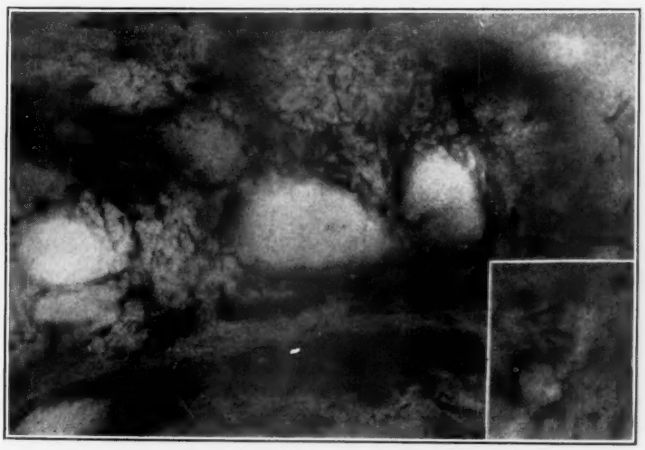

Fig. 7.—Case 7. Extensive infiltration with spirochetes. (×900 mag.) *Inset.* To show coils of individual spirochete. (×1200 mag.)

Dennis, Professor of Parasitology, and D. Berberian, Adjunct Professor of Parasitology, in the medical school of our institution, to both of whom we are indebted for helpful cooperation and suggestions.

It is probable that the majority of spirochetes seen in our cases are syphilitic, but it is realized that our study is still far from complete. Examination of scrapings of the wall of the umbilical vein by the dark-field has been done in a few of our cases, with unsatisfactory results. More exact determination of the variety of spirochetes encountered might be obtained with inoculation into the testes of the rabbit. This has not been done on account of lack of funds. No check up of possible syphilitic perichondritis of the epiphysis of long bones by the roentgen ray has been done by us on living babies. However, twenty cases of stillbirths in this series were autopsied and in each case the bones were free from evidence of osteochondritis. At the Johns Hopkins Hospital 25 per cent of premature babies examined by x-ray, showed signs of syphilis of bone.[18] On the other hand Olsen[19] has reported that of 55 viable healthy children of syphilitic mothers in the Rigs Hospital, Stockholm, who were examined by the x-ray on the second and third days after birth, only one showed osteochondritis.

We feel that while this preliminary report is not complete, there is enough evidence to stimulate further research on this vital problem.

In the 105 cases the average age was 27.3 years, the patients ranging in age from sixteen to fifty-nine years. In 21 per cent of the patients the placenta examined was from the first pregnancy.

A Study of 105 Cases of Positive Findings of Spirochetes in the Placenta

Table I. Present Delivery	
Normal delivery	56
Forceps	14
Embryotomy	3
Cesarean section	11
Version	3
Premature	18
Total	105
The following conditions were found:	
Placenta previa	9
Adherent placenta	7
Polyhydramnios	11

Table II. Condition of Fetus	
Normal	64
Stillborn	32
Neonatal death	9
Total	105
Of these 18 presented abnormalities	

Table III. Weight of Fetus	
2,000 gm. and under	4
2,000-2,500 gm.	9
2,500-3,000 gm.	19
3,000-3,500 gm.	28
3,500 gm. and over	16
Weight not recorded—cases of maceration, embryotomy, etc.	29
Total	105

Table IV. Past History	
Number of primigravidas	22
Number who had had at least one normal child	65
Number who had had at least one abortion	51
Number who had had at least one stillbirth	27
Number who had had at least one neonatal death	5
Number who had had at least one premature birth	3

NORMAL CHILDREN FROM SYPHILITIC MOTHERS

Half of the patients gave a history of at least one previous abortion and one-fourth of the patients a history of repeated abortions. While this figure may seem high, it should be borne in mind that the history of repeated abortions was one of the factors that determined the selection of the placenta for examination.

The past and present history of these 105 patients includes a total of 558 pregnancies. The history of previous pregnancies gave a record of delivery of full-term living children in 61 per cent of the cases, and in the present series of 105 deliveries in which the spirochetes were found in the placenta, no less than 63 per cent were children to all appearances perfectly healthy. Thus we see that the presence of spirochetes in the placenta is perfectly consistent with a seemingly healthy baby.

Of these 558 pregnancies, 238 ended "disastrously" and 320 in apparently normal full-term babies. The "disastrous" pregnancies, as McCord terms them, were: 112 abortions; 48 stillbirths; 23 neonatal deaths; 37 premature labors; 18 full-term babies showing abnormalities. Of the 320 apparently normal children 15 were killed by accidents of birth: 4 placenta previa; 8 dystocia; 3 prolapse of the cord.

Of the 105 patients with the positive findings of spirochetes in the placenta, 30 patients had received antisyphilitic treatment of some sort either before or during the present pregnancy, and 75 patients received no antisyphilitic treatment. The 75 mothers who had not received any treatment gave a history, including the last delivery, of having produced 231 apparently healthy babies and of 160 "disastrous" pregnancies: 74 abortions, 29 stillbirths, 17 neonatal deaths, 22 premature labors, and 18 children showing abnormalities.

Thus it will be seen that the chance of a successful pregnancy in an untreated case of syphilis is only a trifle less than 3 out of 5. The possibility is realized that with some of these mothers syphilis may have been acquired after the birth of the earlier children, but such instances are very few and in the majority of cases the seemingly healthy children are the product of later pregnancies.

VARIED RESULTS FROM THE INFECTION OF THE PLACENTA

While the above proportion of 3 successful pregnancies out of 5 may be true as the average for those 75 cases, it does not hold true for any one individual case. A number of factors may be assumed to influence the course of pregnancy in a syphilitic mother.

The course may vary with:

1. The virulence of the strain of spirochetes.

2. The character of the invasion, whether gradual or abrupt, as well as the massiveness of the infecting dose.

3. It is probable that the character of the invasion is determined by the stage of the disease in the mother, whether it is quiescent or in active stage.

4. The relation of the time of the invasion of the fetus to the vulnerable stages of development of its vital organs.

5. The presence of other complicating lesions such as nephritis.

If the infection is from a mild strain, or the disease is in a quiescent stage, one may anticipate the advent of an apparently normal child, though spirochetes are present in the placenta.

CASE 23.—(Hospital No. 21230.) Patient aged 23, married eight years. Four healthy full-term babies. No abortions. In the last pregnancy the mother's blood gave a two-plus Wassermann; the cord blood was negative. In the present pregnancy only had the mother received antisyphilitic treatment. The placenta showed numerous spirochetes in clumps.

CASE 83.—(Hospital No. 24195.) Patient aged thirty-seven, married twenty years. Five normal full-term children. One abortion. The last delivery was a placenta previa with adherent placenta. The cord blood was negative. Spirochetes were found in the placenta. Had it not been for the presence of a placenta previa there would have been no examination made of the placenta.

CASE 85.—(Hospital No. 24490.) Patient, aged thirty-five, married eighteen years. Has had seven normal full-term deliveries and no abortions. Eleven years previously she had an attack of eclampsia. In the present pregnancy she had an albuminuria of 4-plus, and her blood pressure rose to 210/120. Serodiagnosis of the maternal blood was negative. She aborted a seven months' macerated fetus which had been two months dead in utero. The placenta showed typical spirochetes. Had it not been for the complicating nephritis, the probability is that the child would have been born alive at term.

On the other hand the syphilitic infection may be of such a virulence that pregnancies end in repeated disaster. In one of the cases in this series spirochetes were demonstrated in the placenta of an abortion of four months.

CASE 67.—(Hospital No. 23536.) Patient aged twenty-six, married ten years. Had four premature stillbirths at approximately eight months. Antisyphilitic treatment was then instituted and she gave birth successively to three healthy children at term. The cord blood was negative; nevertheless, the placenta of the last case showed the presence of spirochetes in a few foci only.

Other syphilitic mothers alternate between disaster and success in their pregnancies. We may suppose that in such cases the varying results of pregnancies are determined by the different factors already enumerated.

CASE 91.—(Hospital No. 14562.) Patient aged thirty, married fourteen years. Interspersed with 5 normal full-term children there were one stillbirth, one neonatal death, and one fetus dead at four months. In the last pregnancy only was the patient given a quite inadequate antisyphilitic treatment. The mother's blood gave four-plus Wassermann. The pregnancy was successful in delivering a full-term, apparently normal child. Kahn precipitation test showed four-plus for the cord blood. The placenta showed spirochetes.

CASE 97.—(Hospital No. 24880.) Patient aged twenty-six, married fifteen years. She has had five apparently normal full-term babies, of whom three are now alive. Has had four abortions. Has had no antisyphilitic treatment. In the present pregnancy the serum reaction for both maternal and cord blood was negative. Patient was delivered of a cyanosed eight-month baby. There was polyhydramnios estimated at five liters. The baby lived. A few spirochetes were found in the placenta.

RESULT OF ANTISYPHILITIC TREATMENT

Our records show that in pregnancy with syphilis thorough treatment may be expected to result in successful outcome of the pregnancy in better than 90 per cent of the cases. They also show that an apparently healthy child is frequently born of a syphilitic mother who has

TABLE V. RESULTS OF 30 TREATED AND 75 UNTREATED CASES

	NO. OF CASES	NO. OF PREGNANCIES	APPARENTLY NORMAL	DISASTROUS
Suspected of syphilis and treated	30	167	89	78
Suspected of syphilis and untreated	46	307	163	144
Syphilis suspected during or after delivery; untreated	29	84	68	16
Total	105	558	320	238

NOTE: This table, superficially giving the impression that disastrous pregnancies were more common among the treated, is deceptive. It must be remembered that the patients that were treated were flagrantly syphilitic and were treated for the most part during the last pregnancy only. Tables VI and VII throw a better light on it.

had no treatment at all, and further, that even with thorough antisyphilitic treatment, repeated at each pregnancy, the placenta of an apparently normal child is likely to show the presence of spirochetes.

CASE 20.—(Hospital No. 13136.) Patient aged twenty-seven, married twelve years. The first three children were stillborn at seven, eight, and nine months, respectively. Four years ago during her fourth pregnancy, her blood showed four-plus Wassermann. This time she delivered a full-term child that later died of diphtheria at the age of three. Serodiagnosis of the cord blood was negative. Three years ago after antisyphilitic treatment during her fifth pregnancy, she had another apparently healthy boy. Serodiagnosis of the cord blood was again negative. In the present pregnancy she was again given antisyphilitic treatment. The maternal blood gave negative Kolmer and Kahn reactions. She was delivered of eight-month twins, small, but apparently healthy. Kolmer and Kahn reactions from the cord blood were negative. Examination of the placenta by the Levaditi method showed abundant precipitate and a few spirochetes.

CASE 95.—(Hospital No. 4889.) Patient aged thirty-eight, married fourteen years. Husband gave history of primary chancre and of strongly positive Wassermann. There were three premature deliveries, two at eight and one at seven months, with neonatal death, and one stillbirth years ago. About six years ago she was given antisyphilitic treatment which has been repeated in each subsequent pregnancy, with the result of four successive apparently normal full-term children. In the last pregnancy the maternal and cord blood gave negative serum reactions. The placenta showed spirochetes in a few foci.

TABLE VI. FORTY-SIX PATIENTS WITH SUSPECTED SYPHILIS NOT RECEIVING ANY ANTISYPHILITIC TREATMENT

CASE	PAST HISTORY				SEROLOGY	PRESENT DELIVERY			REMARKS
	ABORTION	PREMATURE	STILLBIRTH	NEONATAL DEATH		DEAD	NORMAL	ABNORMAL	
3	1	–	–	–	–	–	1	–	
4	–	1	–	–	+	1	–	–	Hydramnios
5	3	–	–	1	–	1	1	–	Twins, one neonatal death, oligohydramnios
6	5	–	–	–	–	–	1	–	
9	–	–	–	–	0	–	–	1	Talipes
10	3	–	–	–	+	1	–	–	
13	1	–	–	–	+	1	–	–	Macerated fetus
14	1	–	1	–	0	1	–	–	Large overtime fetus
15	3	–	–	–	–	1	–	–	Anemia, nephrosis, cesarean section
16	1	–	1	–	–	–	–	1	Macular rash on baby's trunk
17	–	–	–	–	–	1	–	–	Anencephalus
18	5	–	–	–	–	–	–	1	Cleft palate
21	2	–	3	–	–	1	–	1	Twins, one dead seven mo. Genu varum
24	2	–	1	–	–	1	–	–	Intrauterine asphyxia
26	1	–	–	3	–	–	1	1	Twins, one normal, one amyotonia
28	1	–	–	–	0	1	1	–	Twins, one normal, one stillbirth
32	1	–	–	–	0	–	–	1	Full term but very small
33	1	–	–	–	0	1	–	–	Intrauterine asphyxia
36	2	–	–	–	–	–	1	–	Albuminuria of pregnancy
37	–	–	–	–	0	–	–	1	Skin lesions, exfoliation
40	2	–	–	–	0	–	1	–	History of one hydatiform mole
41	3	–	–	–	–	–	–	1	Mongolian idiocy
45	2	–	1	–	0	–	–	1	Clubbed feet
46	5	–	1	–	+	–	1	–	Developed melena
47	–	–	–	–	++++	–	1	–	Cord Wassermann strongly positive
49	–	–	2	–	–	1	–	–	Macerated fetus
50	1	–	–	–	–	1	–	–	Prolapse of cord
51	3	–	–	–	0	1	–	–	Abortion at 4 months
52	1	–	1	–	++++	–	1	–	Cord Wassermann ++++
53	–	–	1	–	0	1	–	–	Eclampsia, intrauterine asphyxia
54	1	–	1	1	++++	1	–	–	Polyhydramnios cord ++++
58	1	–	–	–	–	1	–	–	Premature, died third day
60	–	–	1	–	0	1	–	–	Macerated
63	–	–	–	–	–	–	–	1	Osteogenesis imperfecta
64	–	–	1	–	–	1	–	–	Left tentorial tear
70	–	–	–	5	–	–	1	–	Died third day, acute purulent colitis
79	1	–	–	–	–	1	–	–	Macerated, father – – – – Wassermann
80	–	–	–	–	–	–	1	–	History of positive Wassermann
81	1	–	–	–	0	1	–	–	Bleeding 40 days before abortion
86	–	–	1	–	–	–	1	–	Cesarean for narrow pelvis
87	–	–	–	–	–	–	–	1	Cretinlike, mother enlarged thyroid
89	3	–	–	–	–	–	1	–	Prolapse of cord
92	1	–	–	–	–	1	–	–	Placenta previa, premature 8 mo.
97	4	–	–	–	–	–	1	–	Polyhydramnios
98	–	–	–	–	+	–	1	–	Manual extraction of placenta
101	1	–	–	–	0	1	–	–	Central placenta previa 8 mo.
46	63	1	16	10	25 neg. 8 pos. 13 not done	24	15	11	

TABLE VII. THIRTY PATIENTS WITH SUSPECTED SYPHILIS RECEIVING ANTISYPHILITIC TREATMENT DURING THE LAST PREGNANCY FOR THE MOST PART

CASE	PAST HISTORY				SEROLOGY	TREATMENT		PRESENT DELIVERY			REMARKS
	ABORTION	PREMATURE	STILLBIRTH	NEONATAL DEATH		PAST	PRESENT	DEAD	LIVING		
									NORMAL	ABNORMAL	
1	4	–	–	1	–	–	+	1	–	1	Twins, living had erythematous rash; dead transposition of viscera
2	4	–	–	–	++++	+	+	–	1	–	Mother bismuth line on gum
7	1	–	–	–	+++	–	+	–	1	–	
8	–	2	–	–	–	+	–	–	1	–	
11	1	–	–	–	–	–	+	–	1	–	
19	–	4	–	4	0	–	+	–	1	–	Baby had repeated attacks of cyanosis, atelectasis, recovered
20	–	3	3	–	–	+	+	–	2	–	Twins, healthy, 8 months
23	–	–	–	–	++	–	+	–	1	–	
27	–	–	1	–	–	–	+	–	1	–	Cesarean section
38	–	–	–	–	++++	+	+	–	1	–	
42	–	–	1	–	–	+	+	–	1	–	Father had positive serology
43	1	–	–	–	++++	–	+	–	1	–	Cord Wassermann ++++
44	4	–	–	–	–	–	+	–	1	–	Premature at 8 months
55	3	1	1	–	–	–	+	–	1	–	
59	3	–	–	–	–	+	+	1	–	–	Abortion at fifth month
61	2	–	–	–	–	+	–	–	1	–	
67	1	–	4	–	0	+	–	–	1	–	
71	4	–	1	–	0	–	+	–	1	–	
74	1	–	2	–	+	+	+	–	1	–	Cesarean section
75	4	–	–	–	+	–	+	–	1	–	
76	2	–	–	–	–	+	+	–	1	–	
77	–	–	2	–	–	+	+	–	1	–	
78	–	2	–	–	–	+	–	–	1	–	
91	1	1	1	1	++++	–	+	–	1	–	Cord Kahn ++
93	2	–	–	–	+	+	+	–	1	–	
95	–	3	1	–	–	+	+	–	1	–	
96	3	–	–	–	–	+	+	–	1	–	
99	–	–	–	–	–	+	+	–	1	–	
103	–	–	3	–	–	+	–	–	1	–	Child lived 2 days. Bronchopneumonia
105	2	–	1	–	0	+	+	–	1	–	Cesarean section
30	43	16	21	6	17 neg. 9 pos. 4 not made			2	29	1	

NOTE: A synopsis of this and the previous table shows the benefit of antisyphilitic treatment. In Table VI, of 46 untreated patients there were 50 babies (four twins), 24 dead and 26 living (16 being normal and 10 showing abnormalities). In Table VII of 30 treated patients there were 32 babies (two twins), 2 dead and 30 living (29 being normal and only one showing abnormality). Again note the higher percentage of serologically positive reactions among the treated cases than among the untreated, showing that the treated cases were the more strongly syphilitic.

SEROLOGIC FINDINGS IN CASES OF POSITIVE FINDING OF SPIROCHETES IN THE PLACENTA

Less than one-third of these cases gave any degree of positive serum reaction from the mother's blood, and these were all cases in which the spirochetes were demonstrated in the placenta. This is in accord with the statement of Gellhorn[20] that after five years of syphilis, whether treated or untreated, 60 per cent or more of the patients gave negative serologic tests.

TABLE VIII

	NEGATIVE	+	POSITIVE			
			++	+++	++++	TOTAL
Maternal blood in 75 cases	54	12	2	1	6	21
Per cent for maternal blood	72	16⅕	2⅘	1⅕	8	28
Cord blood in 71 cases	64	2	1	1	3	7
Per cent for fetal blood	90	3⅕	1⅖	1⅖	4⅖	10

NOTE: In about half the cases the serum diagnosis was made by the Kolmer-Wassermann method as well as by the Kahn. In the others the Kahn alone was used, but wherever this was positive the Kolmer was also done. Usually the results of the two methods were the same. In 4 cases only the Kahn was positive and the Kolmer negative, and these were recorded as positive.

From the cord blood only 10 per cent of the cases gave positive reactions, and these only in cases where the mother's blood also gave a positive reaction. Where a positive reaction from the cord blood can be obtained in only 10 per cent of the cases in which the spirochetes are demonstrable in the placenta, the futility of reliance on the examination of the blood of the newborn is apparent. It is quite probable that fetal blood itself gives no positive Wassermann reaction and that the few instances where positive reactions are obtained occur only in those cases where the trauma of delivery has permitted a leakage of maternal blood through the placenta into the fetal circulation.[21]

THE RELATION BETWEEN THE CLINICAL DIAGNOSIS OF SYPHILIS AND THE DEMONSTRATION OF THE SPIROCHETES IN THE PLACENTA

The clinical diagnosis of syphilis was judged by the presence of repeated abortions, stillbirths, or premature deliveries; by positive serodiagnosis; by previous history of syphilis; or by the presence of physical signs. The unsuspected cases include cases of single abortion, of placenta previa, of cesarean section, of stillbirths and of deformities

TABLE IX

CLINICAL DIAGNOSIS		SPIROCHETES PRESENT IN PLACENTA		
Total number of cases		Few	Moderate	Numerous
Syphilis probable	63	22	26	15
Syphilis questionable	13	3	8	2
Syphilis unsuspected before delivery	29	10	17	2
Total	105	35	51	19

in the fetus. The histologic condition of the placenta was not taken into consideration in the composition of this table.

During the period in which the 105 cases with positive findings were under investigation, forty other cases were studied in which negative results were reported. In 11 of these the clinical history made a diagnosis of syphilis probable, but no spirochetes were found in the placenta. In the other 29 cases, where there was no presumption of syphilis, the findings were negative.

SUMMARY

1. The finding of spirochetes in the placenta in 105 cases is recorded.

2. Spirochetes can be found in the placenta of the syphilitic newborn in sufficient frequency to justify the search for them in suspicious cases.

3. The search should be made after Levaditi infiltration in portions of the placenta which give an indication of their presence by the presence of pale yellow foci surrounded by dark granular peripheries.

4. In 391 pregnancies from 75 syphilitic mothers who were untreated and with whom there were demonstrable spirochetes in the placenta of the last delivery, an apparently healthy baby was produced in three out of five pregnancies.

5. The fact that the newborn baby appears to be healthy does not indicate the absence of syphilis.

6. The successful termination of pregnancy after antisyphilitic treatment does not denote the absence of spirochetes from the placenta.

7. The histopathologic appearance of a placenta containing spirochetes is discussed.

8. Thorough antisyphilitic treatment, while it may not cause the disappearance of spirochetes from the placenta, is nonetheless indicated as it assures about 90 per cent apparently healthy full-term babies.

The authors desire to express their appreciation of assistance in the arrangement and presentation of the text, kindly rendered by Dr. Amin A. Kheirallah, Lecturer in Surgery in the American University of Beirut.

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CHRONIC UTERINE DISTENTION AND ITS RELATION TO THE END OF GESTATION*

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IN 1913, Dickinson and Smith published a paper[2] entitled, "The Treatment of Anteflexion, Defective Function, and Sterility by Glass or Silver Stems." One of their concluding remarks contains the statement that insertion of stem pessaries into the cervical canal "may develop an infantile uterus, restore the organ of premature menopause or atrophy, and bring back superinvolution to a normal condition. Ovarian enlargement will take place *pari passu* with the uterine enlargement."

This observation, made when the nature and number of ovarian hormones was unknown, has remained unnoticed for nearly twenty-five years without attempt by physiologists to learn more of the effects of uterine distention upon reproductive functions. The present paper is a beginning, it is hoped, of what may prove to be recognition of the importance of uterine distention in certain phases of uterine function. At the same time, it may serve to reawaken the interest of the clinician in the possibilities of chronic distention in conjunction with attempts at modern ovarian hormone therapy.

I. HISTORICAL CONSIDERATIONS

Some importance has been attached in the past by clinicians to uterine distention. Thus the fact is well known that the size of a uterus in advanced ectopic pregnancy is smaller than is that of another uterus at a comparable stage of normal pregnancy. On the experimental side, Blair-Bell and Hick, in 1909, compared[1] the similarity of structure of the myometrium in the pregnant uterus before and after evacuation to the structure of the myometrium in experimentally produced hydrometria before and after emptying the uterus. Aside from this work which seems to have been little noticed in the intervening years, the question of the relation of uterine distention to uterine function has been generally neglected until recently.

In 1929, Knaus[5a] showed that an occupied horn of a rabbit's uterus in unilateral pregnancy is significantly larger than the sterile horn in the same animal. In such a case, both uterine cornua are exposed to

*The experiments on the effects of graded uterine distention which are summarized and discussed together for the first time in this paper, were carried out with the aid of a grant of money to the writer from the Committee for Research in Problems of Sex, of the National Research Council. Acknowledgment to that Committee is gratefully made.

the same hormone environment. This observation has been confirmed on several recent occasions (rabbit, 3 and 7; cat, 6) and serves to extend to the rabbit (and cat) the older observation of the clinician with respect to the size of the uterus in ectopic pregnancy. Another advance in our appreciation of the rôle of distention alone (in contrast to the more complicated conditions associated with pregnancy) was made in 1929 by van Dyke and Gustavson[14] when they observed that if rolled rubber dam is inserted into a uterine horn during pseudopregnancy, local uterine growth takes place at the site of distention. This observation, made in connection with experiments designed for another purpose, has escaped the notice of other investigators until now. Another finding which is comparable to that of van Dyke and Gustavson has been reported recently by Markee, Wells and Hinsey.[7] These investigators noted that uterine growth takes place in a uterine horn distended with fluid secreted by its own endometrial glands. This observation differs from the earlier ones of Blair-Bell and Hick who stressed only the histologic features of experimental hydrometria in that this newer work established with quantitative measurements the fact that uterine enlargement occurred.

These considerations demonstrate that there is a local factor favoring uterine growth at a site of distention, but they do not show that this factor operates in the absence of ovarian hormones since the ovaries were present and functioning in each instance. Consequently, these experimental data do not constitute a confirmation of the earlier clinical work of Dickinson and Smith in whose observation uterine enlargement, ovarian enlargement, and even restoration of periodic bleeding resulted from uterine distention during a hypohormonic (amenorrheic) state. Experimental confirmation has lately been forthcoming as regards their observation pertaining to uterine enlargement, however. This has been obtained as a result of uterine distention in untreated, ovariectomized rabbits.[12]

II. EFFECTS OF GRADED UTERINE DISTENTION UNDER VARIOUS HORMONIC CONDITIONS

Experimental confirmation of the clinical observations of Dickinson and Smith was obtained in experiments of the following type. Suitable rabbits were ovariectomized and at the end of one week, paraffin pellets (melting point 56° C.) were inserted into the uterus per vaginam and anchored in place. Two weeks later these distention sites were taken, along with a segment of undistended, untouched uterus. With the aid of an appropriate technic,[12] the percentage increase (growth) of the various distention sites was determined. The extent of this growth was then correlated with the degree of distention (i.e., the intensity of stimulation) in each instance. As a result, the curve of uterine growth to graded degrees of distention in untreated, ovariec-

tomized rabbits was established.[12] This curve shows that if a pellet is to be an adequate stimulus for growth it must be more than half the size of the undistended uterus yet less than twice its size. The largest growth responses are obtained when the uterus and pellet are of approximately equal sizes.

As a consequence of the establishment of these relationships in the absence of ovarian hormones, a base line was obtained to which the separate actions of estrin and progestin on the distention-growth response could be referred. Accordingly, two new sets of experiments were performed to evaluate quantitatively the effects of estrin and progestin respectively in uterine growth resulting from distention.

In one group of experiments[9] estrin was given before and during the period in which the pellets were inserted. It was found that chronic uterine distention in these estrinized rabbits resulted in a reduction of the capacity of the uterus to grow. This reduced growth capacity of the tissues was more pronounced in the endometrium than in the myometrium and in both tissues the growth responses were much less than in the untreated, ovariectomized rabbit. This effect was explained on the basis of a diminished blood supply at the site of distention, resulting from the increased tonicity and contractility of the myometrium under the influence of estrin.

The second group of experiments[10] was designed to show the effect of progestin upon the distention-growth response of the uterus. The results showed that in mature, ovariectomized rabbits treated with progestin, growth of the tissues about the pellets takes place. The form of the growth curve in these experiments is very similar to that obtained in untreated, ovariectomized rabbits. The limits of the curve are different, however, for it was found that if the pellets are to be an effective stimulus, they must be more than two-thirds the size of the undistended uterus (instead of half, as in untreated rabbits), yet less than four times the size of the undistended uterus (instead of twice the size, as in untreated rabbits). Optimal growth responses are obtained in progestin-treated rabbits when the pellets are about twice the size of the undistended uterus, in contrast to the untreated rabbits in which optimal growth is observed when the pellet and uterus are of equal size.

Briefly summarized, these results show that when progestin is acting upon the distended uterus, the degree of distention required to produce a given amount of growth must be appreciably greater than that required to have the same effect in untreated, ovariectomized rabbits. This fact has been ascribed to the decreased tonicity of the myometrium resulting from the action of progestin upon it. As a consequence, it appears that the increased distention is required to produce an appropriate degree of tension which is essential for the distention-growth response. As mentioned above in connection with the effects of estrin,

however, if the tension becomes too great the growth-response is affected adversely, especially in the endometrium, through impairment of the blood supply.

III. THE RÔLE OF UTERINE DISTENTION IN PREGNANT RABBITS

The physiologic significance of the facts described above is concerned with changes occurring during gestation since distention is an ever changing factor throughout most of pregnancy. Fortunately, suitable data on the growth of the uterus and of the products of conception are available in a recent paper by Professor Hammond, of Cambridge, in the Russian Journal, *Transactions on the Dynamics of Development*.[3] The data given are for the rabbit, thus enabling one to make analysis of them directly from the standpoint of the distention-growth responses described above, which were likewise obtained in the rabbit. Markee, Wells and Hinsey[7] have also published quantitative data on uterine growth during pregnancy in rabbits and these have been used to support Hammond's data.

Experimental Data Available for Study.—In Table I, I have modified some data from Tables I and VIII in the paper by Hammond. The changes made are first, to take the sum of the weights of the fetuses, fetal fluids, fetal placentas and membranes, and the maternal placentas. Values for these are given separately by Hammond for selected days of pregnancy. Since together they constitute the distention-mass in pregnancy, they have been grouped in the present paper under the term "distention-mass." The second modification of Hammond's data is found in the column headed "sterile horn mass." Hammond quotes weights (his Table VII) for a single sterile horn in unilateral pregnancies, while the weights of the gravid uteri are given in Table I for two uterine cornua. It is desirable to know what part of the

TABLE I. WEIGHTS OF THE REPRODUCTIVE ORGANS OF THE RABBIT AND OF THE PRODUCTS OF CONCEPTION AT DIFFERENT STAGES OF PREGNANCY (FROM HAMMOND). SEE FIG. 1

DAYS OF PREGNANCY	AVERAGE NUMBER OF EMBRYOS	DISTENTION-MASS: PRODUCTS OF CONCEPTION	MASS OF THE GRAVID UTERINE TISSUES	MASS OF STERILE HORN EQUIVALENTS
		(GM.)	(GM.)	(GM.)
0	0.0	0	6.50	6.50
8	9.0	0.284	7.80	
12	9.7	2.673	15.54	
16	6.2	6.73	16.54	6.46
20	7.5	11.92	27.17	
24	5.2	28.35	35.68	15.00
29	4.7	52.01	32.61	16.90
30	5.3	58.11	37.05	17.68
32	4.8	75.44	48.81	20.58

gravid horn mass at each of the stages of pregnancy studied is equivalent to the sterile horn mass. This may be done by multiplying by

two the weights given for the single sterile horns, or by dividing by two the weights given for the gravid uterine horns. The former has been done in this paper. Such a procedure is permissible for two reasons. In the first place, Markee, Wells and Hinsey have shown that in a nonpregnant rabbit the sizes of the two uterine cornua are not appreciably different. In the second place, it is allowable because of the fact that Hammond quotes average weights for a number of cornua at the several stages of pregnancy studied, and he has used rabbits which "have been standardized as far as possible by using inbred strains which were selected for a definite size and fertility."

The data which are available, therefore, and are useful in the considerations which follow are: (1) the days of pregnancy; (2) the mass of the products of conception; (3) the mass of the gravid uterine tissues; (4) the mass of the sterile horn equivalent in each gravid

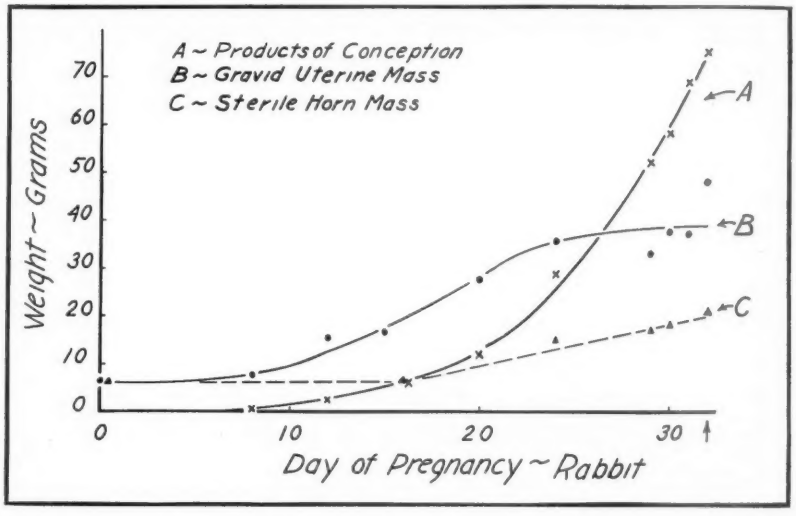

Fig. 1.—Chart showing growth during pregnancy of the products of conception (*A*), the gravid uterus (*B*), and of the sterile horn mass (*C*) calculated from unilateral pregnancies. Data from Hammond.

uterus; and (5) the average number of fetuses in each group. The last is important because Hammond shows that the number of fetuses in a uterine horn affects the amount of growth taking place in it.

Uterine Growth at Different Stages of Pregnancy.—With this information one may plot in a graph the weights of the products of conception (distention-mass) and the weights of the gravid uterine tissues on the different days of gestation.* Fig. 1 shows such a correlation. In addition to these two curves, the weights of the sterile horn equivalents are shown in Curve C.

In this graph, attention is called to two features in particular. In the first place, during the latter third of pregnancy in the rabbit, the

*Pregnancy in the rabbit lasts thirty-two days.

rate of growth of the gravid uterus decreases whereas that of the products of conception increases to a maximum. Thus, from the twentieth day on, an increasing disproportion exists between the size of the distention-mass and the size of the uterus which surrounds it. In other words, the gravid uterus approaches the limit of its capacity to grow, for the conditions existing near the end of gestation. More will be said of this correlation below.

In the second place, Curve C shows that between the sixteenth and the twenty-fourth day, the sterile horn of a unilateral pregnancy commences to increase in size. Hammond points out that this takes place about the twentieth day. The second correlation to be noted, therefore, is that as the curve for the distended, gravid uterine horn (Curve B) becomes flattened, that for an undistended horn exposed to the same hormonic influences begins to increase (Curve C). These facts are elucidated still further by an analysis of the data in the manner employed in the pellet work described above. Such an analysis is made as follows.

Uterine Growth During Pregnancy Attributable to the Distention-Mass.—With Hammond's data arranged as in Table I, one may easily compute the percentage increase of the mass of the gravid horn (Curve B, Fig. 1) over the mass of the sterile horn equivalent (Curve C, Fig. 1) in each of the gravid horn-mass values for any day of pregnancy. This percentage increase represents, as in the pellet work described above, the amount of growth which is attributable to the distention-mass. This is so, since the sterile horn equivalent is comprised of the mass of tissue present at the outset of pregnancy, plus the increment resulting from growth due to hormonic influences. These values have been calculated and are indicated in Fig. 2.

In this graph it was necessary to treat the data according to the number of fetuses present, because, as Hammond observes, this affects the amount of uterine growth. Accordingly, what is shown in Curve A of Fig. 2 is a family of curves of varying magnitude but having similar contours. Taken together, they show that uterine growth which is attributable to the distention-mass increases up to the twentieth day, and after this time it decreases somewhat, as shown by the flattening of the curve. A similar curve has been obtained with the data of Markee, Wells and Hinsey.[7]

It may be noted in passing that the growth responses obtained up to about the twentieth day are compatible with the data obtained in the distention-growth work in progestin-treated rabbits, described above.

The fact that after the twentieth day the growth due to distention decreases, taken in consideration with the fact that estrin exerts an inhibiting effect upon the distention-growth response, shows that the

hormonic influence of estrin is becoming increasingly pronounced after the twentieth day. Other evidence dealing with the reactivity of the uterus at this time might also be adduced if other support for this conclusion were necessary.[5b, 11, 13] Parenthetically it may be noted that estrin is present in increasing amount in the blood and urine of the human being up to the time of parturition.[8]

This conclusion raises a point, however, which must be considered before any special significance may be attached to it. If one calculates the percentage increase in the mass of sterile horn equivalents on the different days of pregnancy over the mass of uterine tissues present at the start of pregnancy, he will find (as also shown in Fig. 1) that this increases from about day twenty until the end of gestation. This is shown by Curve B of Fig. 2. Since this growth of the sterile horn is attributable to hormone influences, as shown above, it is in all prob-

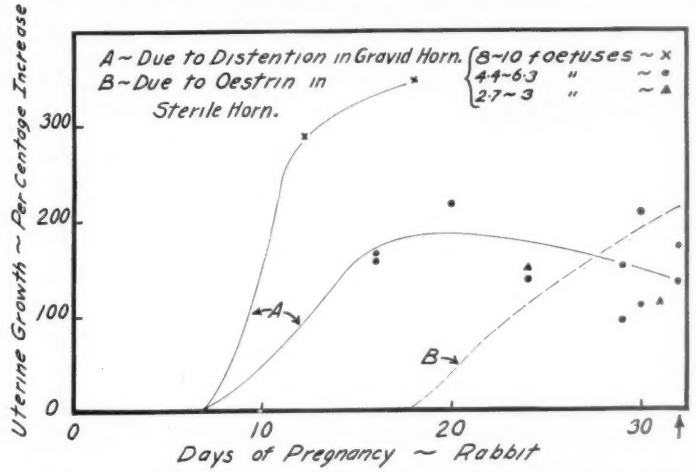

Fig. 2.—Chart showing the growth (percentage increase) during pregnancy of the gravid uterus (*A*), which is attributable to the distention-mass (products of conception), and the growth (percentage increase) of the sterile horn mass in unilateral pregnancies (*B*). See text for mode of calculation and significance.

ability associated with a rise of estrin which, as also pointed out above, is limiting the distention-growth response of the gravid uterus at this time.

IV. ESSENTIAL PHYSIOLOGIC CONDITIONS NECESSARY FOR THE ONSET OF LABOR

The important question arises, therefore: Does the uterine growth which is attributable to hormonic influences (specifically, estrin; Curve B, Fig. 2) after the twentieth day compensate for the diminishing influence of the distention-mass on uterine growth, shown in Curve A of Fig. 2? If it does compensate, then the uterus will continue to grow and in so doing will accommodate more readily the growing conception-mass; if not, it is inevitable that the disproportion between

the growth of the uterus and the growth of the fetus (shown in Fig. 1) will result in a gradual but ever increasing intrauterine tension. This condition in association with the fact that estrin increases rhythmic uterine contractility[8] would result in a condition which is incompatible with retention of the uterine contents. Moreover, the increasing tension on the uterine wall would improve, up to a certain point, the efficiency and forcefulness of the developing uterine contractions.

If the answer to this question is known, therefore, it may be possible to regard the conditions leading up to the onset of labor as a gradual, accelerating convergence of a number of influences, hormonic, nutritional and physical, which must in the normal course of events result in evacuation of the uterus. Fortunately, it is possible to supply the answer to this question with data given in Table I. This is done in the following manner:

Hammond's data show that during the first week of gestation in the rabbit, no appreciable increase in the size of the uterus occurs (Curve B, Fig. 1); the growth at this time is almost entirely that of

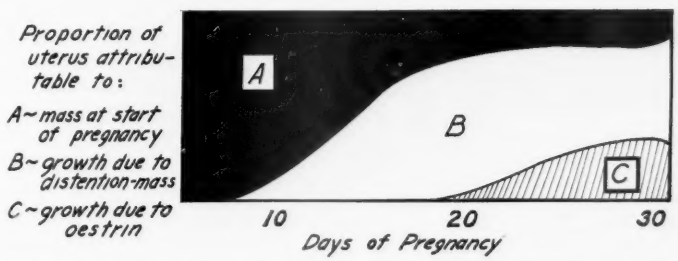

Fig. 3.—Chart showing for each phase of pregnancy the proportion of the gravid uterus which is attributable to the tissues present at the start of pregnancy (*A*), to the growth-increment attributable to the distention-mass (*B*), and to the growth increment attributable to the action of estrin (*C*). See text.

progressive differentiation of certain tissues. Between the eighth and twelfth days (by which time the ova have become attached and commence to grow rapidly) definite uterine enlargement begins. The problem that must be solved is to ascertain at the several stages of pregnancy (16, 24, 29, 30, 32 days) that part of the gravid uterine mass (taken as 100 per cent) which accounts for the tissues present at the start of pregnancy; that proportion which accounts for the growth-increment resulting from the presence of the products of conception, and that proportion which accounts for the growth-increment due to hormonic influences. This has been done and the values are given in Table II. Fig. 3 is based on these calculations from Hammond's data obtained from rabbits having an average of 4.7 to 6.2 fetuses each.

This chart shows that until about the eighteenth day, uterine enlargement takes place almost entirely as a result of the presence of the distention-mass as allowed (or favored) by the action of progestin.

From this time on, the proportion of the gravid uterus which is attributable to hormonic (estrin, see above) influences increases as the proportion due to distention becomes about constant, *pari passu*.

It is highly significant (and this is in answer to the question asked above) that at no time does the growth-increment which may be assigned to hormonic influences equal the growth-increment which is attributable to the presence of the distention-mass. At most, according to these data, it exerts about half the effect that the distention-

TABLE II. PROPORTION OF GRAVID UTERINE TISSUES ATTRIBUTABLE (1) TO THE TISSUES PRESENT AT THE START OF PREGNANCY; (2) TO GROWTH RESULTING FROM HORMONE INFLUENCES; AND (3) TO GROWTH RESULTING FROM THE DISTENTION-MASS (PRODUCTS OF CONCEPTION). BASED ON HAMMOND'S DATA FOR PREGNANCIES WITH 4.7 TO 6.3 FETUSES. SEE FIG. 3

DAYS OF PREGNANCY	PER CENT ATTRIBUTABLE TO ORIGINAL TISSUES	PER CENT ATTRIBUTABLE TO HORMONE INFLUENCE	PER CENT ATTRIBUTABLE TO DISTENTION
0	100.0	0.0	0.0
16	39.3	0.0	60.7
24	18.2	23.8	58.0
29	19.9	31.9	48.2
30	17.6	33.8	48.6
32	13.3	28.9	57.8

mass factor does in uterine growth, in the latter part of pregnancy. Clearly, therefore, the reason why uterine growth at the end of gestation in the rabbit does not keep pace with that of the fetus and its associated parts is due, first, to the limiting action of estrin upon the distention-growth response of the uterus and, second, to the fact that estrin is relatively ineffective as a direct growth-promoting stimulus to the gravid uterus at this time. One must also conclude that the uterus has about reached its limit of capacity to grow at the end of gestation for the conditions obtaining at that time. New or different conditions conceivably may advance the time when the limit of uterine growth is obtained and result in abortion or premature delivery, or they may retard it and give rise to prolongation of pregnancy.

The above conclusion depends upon the assumption that the gravid uterine tissues utilize the available hormones to the same extent as do the uterine tissues in the sterile horn of a unilateral pregnancy. This may or may not be true, however. If the gravid tissues utilize the available hormones to a less extent than do the sterile horn tissues, then the cogency of the argument advanced above is enhanced because the reduced growth-promoting effect of distention after the twentieth day would be proportionately greater. On the other hand, the gravid tissues may grow relatively more because of the hormone; even so the growth which takes place is still insufficient to prevent the increasing disproportion in the growth rates of the uterus and of the products of conception, as shown by Curves A and B in Fig. 1. The main conclusions derived from the foregoing considerations are essentially valid, therefore.

It is clear from the foregoing considerations that the theory advanced in the sequel to the question stated above has an experimental basis of fact. It is to be emphasized, however, that this theory is not all-exclusive, for it does not preclude the probability of a multiplicity of other factors also being concerned in the normal termination of pregnancy. The theory has merit, however, since it defines in quantitative terms for the rabbit a number of growth-promoting factors and growth-limiting factors, the proper association of which must be achieved if labor is to begin.* In addition, emphasis has been laid upon the fact that the immediate cause of the limitation of uterine growth at this time (estrin) is also the stimulus for rhythmic uterine contractility which develops concurrently with the growth changes described above. By virtue of the combined effects of these conditions a means is thereby provided by which the developing rhythmic contractions of the uterus are rendered physiologically more effective for the expulsive functions they ultimately subserve at the time of parturition.

V. SPONTANEOUS ABORTION IN RELATION TO THE ONSET OF LABOR

To say that this theory provides a basis upon which the mechanisms associated with spontaneous abortion may be explained goes beyond the established facts. There are, however, several features in common between this condition and those described above which are necessary for the onset of labor. Chief among these is the fact that spontaneous abortion in the human is preceded for some time by an abnormal rise in the blood and urine levels of estrin.[4] The presence of this hormone must necessarily preclude further uterine growth resulting from distention, on the basis of what has been said above. Such a condition is, as we have seen, one of major importance in determining the capacity of the uterus to accommodate the ovum. Clearly, therefore, increased intrauterine tension will develop and this will lead to death of the fetus through impoverishment of its nutritional supply, owing to interference with the vascular bed of the placenta. In the absence of additional facts it is unwise to speculate further in this connection. Enough information is available, however, to indicate that the conditions leading to spontaneous abortion may have a physiologic basis akin to those which lead to labor.

SUMMARY

At the outset, a review of the local physiologic effects of chronic uterine distention under various hormone conditions is given. It is shown that uterine growth resulting from distention takes place in

*In mammals with the so-called *uterus simplex*, orientation of the physical aspects of parturition would be relatively more important than in the rabbit, which has a bicornuate uterus. See (8) for discussion of the physical changes that must take place.

untreated, ovariectomized rabbits and in rabbits under the influence of progestin. When estrin is the predominant hormone, the capacity of the distended uterus to grow is appreciably reduced.

With these facts as a basis, analysis is made of Hammond's data on uterine and fetal weights at different stages of gestation in the rabbit. It is shown that a disproportion exists between the growth increments of the fetuses and of the uterus in the last third of pregnancy, the former growing much more rapidly than the latter. It is further shown that the reason for the increasing disproportion of these growth rates is due largely if not entirely to an increase in the influence of the hormone, estrin. Mention is also made of the fact that estrin is the hormone demonstrated to have the property of imparting rhythmic contractility to the uterus. As a consequence of the limitation of the capacity of the uterus to grow, along with the continued increase in the size of its contents, it is pointed out that the developing rhythmic uterine contractions are rendered increasingly more efficient and forceful. The theory is advanced, therefore, that these physiologic conditions are the underlying factors which are essential to the onset of labor, and the commencement of parturition is the result of a gradual and accelerating convergence of these factors, bringing about nutritional changes and an appropriate physical orientation of the fetuses. Finally, the common physiologic basis between these conditions favoring the onset of labor and those which may be responsible for spontaneous abortion is discussed.

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THE EFFECT OF LONG-CONTINUED LARGE DOSES OF FOLLICLE HORMONE UPON THE UTERUS OF THE RAT

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AS I have shown in previous studies, it is possible to eliminate certain functions of the anterior lobe of the pituitary by long-continued treatment with follicle hormone. If one treats young rats for a number of weeks with dimenformon (estradiolbenzoate), the following symptoms appear: the growth of the sexual organs ceases so that the scrotum disappears entirely, the testicles are no longer palpable, and the penis becomes small. After several weeks the difference between the weight of the testicles and those of the controls amounts to 95 per cent. Spermatogenesis disappears. The ovaries become so small that they are hardly visible to the naked eye. In serial sections occasional enlarged follicles are seen, never a corpus luteum. The same changes can be effected in birds. The coxcomb disappears, the testicles remain atrophic, and spermatogenesis ceases entirely. Secondarily, disturbances of growth appear. The rats and fowl are retarded in growth, finally all growth disappears so that, for example, the subjects of the experiment are 26 per cent smaller and 60 per cent lighter than the controls. It is possible, then, by chronic treatment with follicle hormone to raise eunuchoid dwarf rats and dwarf fowl. The dwarfism is proportional, the head is smaller, narrower, the breast circumference less, the tail shorter, the internal organs and bones smaller and lighter. Characteristic changes of the bones are easily recognizable in the x-ray picture. (I described these elsewhere in detail.[1]) The above-named effects are ascribable to the elimination of the influence of the secretion of the anterior lobe of the pituitary by the chronic treatment with follicle hormone, specifically the growth hormone and the gonadotropic hormone. That it is really the elimination of these hormones is evidenced by the fact that one can again stimulate growth of these dwarf rats by the exhibition of Evans' growth hormone. To a certain extent the atrophy of the genitals also may be halted by the simultaneous treatment with dimenformon-gonadotropic hormone. In this case, only the growth of the animal is disturbed and dwarfs with normal genitalia are produced.

Follicle hormone only eliminates certain functions. The thyreotropic and parathyreotropic hormones are not influenced, probably also not the

corticotropic. Thus to a certain extent the follicle hormone produces a biologic partial resection of the anterior lobe of the pituitary. Analysis of the pituitary of dwarf rats has shown that the content of gonadotropic hormone is not less than those of controls. From that it follows that it is not the production of gonadotropic hormone that is checked by follicle hormone, but only the delivery of the produced hormone into the blood stream. Follicle hormone then, causes a partial blockade of the anterior lobe of the pituitary. If this treatment with follicle hormone is continued for seven months, we note an interesting result. The pituitary changes into a tumor which can attain a size twenty-five times that of normal. The animals die with symptoms of intracranial pressure. So far I have been able to produce eighteen such tumors of the pituitary which I shall report in detail elsewhere.

In the present study, we are dealing with the effect of chronic treatment with hormone on the uterus. This effect is not a central one as the result of the inhibition of the pituitary, but the expression of a local effect of follicle hormone on the uterus itself. I feel particularly honored to contribute the results of this investigation to the volume dedicated to Dr. Robert T. Frank, to whom we are indebted for so many significant researches in the field of the follicle hormone.

In these experiments the rats received for a number of weeks doses of 5,000 M.U. dimenformon twice a week. It must be made clear that these are enormous, totally unphysiologic doses. But if we can attain definite reactions in the uterus, certain conclusions in reference to pathology are justifiable by analogy, for the ovary may produce very large unphysiologic amounts of follicle hormone for considerable periods of time, for instance, in certain disturbances which I have described under the heading of polyhormonal syndromes.[2] The best known of these is glandular cystic hyperplasia of the endometrium. It will be shown below that by chronic treatment with follicle hormone the endometrium may be destroyed and the uterus disintegrated by suppuration.

Partial inhibition of the anterior lobe function can be accomplished regularly by follicle hormone; in 140 test rats there was not a single failure. In the case of the uterus the situation is entirely different.* Under the same experimental conditions there may be no effect on the uterus of one rat and a high grade disturbance of the uterus of another. The uteri of various animals react differently; a fact which I report, but cannot explain. Space prevents me from describing the individual experiments, and I will, therefore, simply mention the quite characteristic changes. In practically all the experiments there was an extensive leucocytic infiltration of the mucosa particularly a subepithelial

*I should like to thank Dr. Joel and Dr. Karplus for their invaluable assistance in this work.

wall of eosinophiles, some of which wandered in numbers into the uterine cavity. I emphasize this because I believe that an inflammatory factor plays a considerable rôle in the changes described. It is characteristic that the epithelium can show considerable variation in the same section. There are stretches of normal single layered cylindrical epithelium; else-

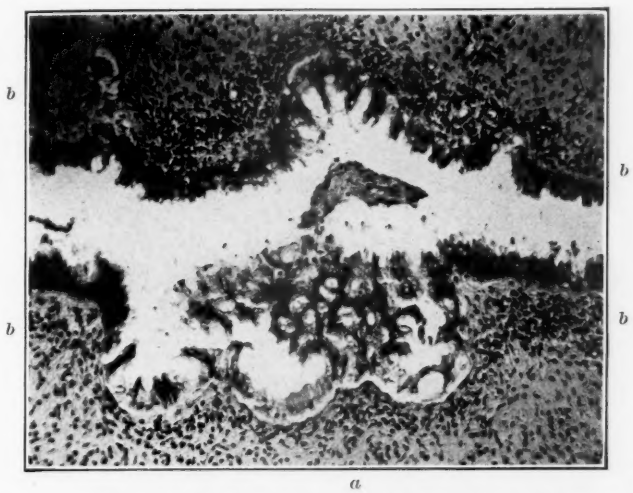

Fig. 1.—Cross-section of the uterus, 150 magnifications. The mucosa lifted off in areas. Hydropic cells without visible cell borders (*a*). An encircling ring of subepithelial eosinophiles (*b*). Some transmigration into the lumen.

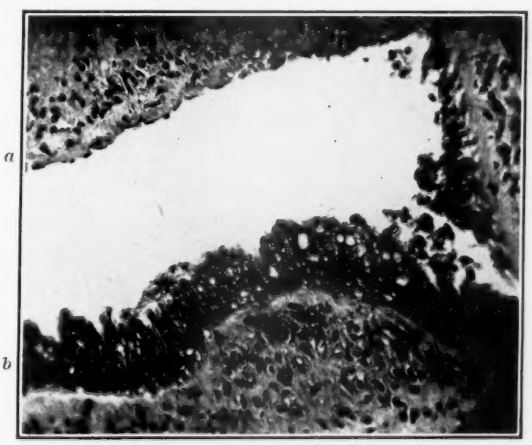

Fig. 2.—Cross-section of the uterus, 270 magnifications. Areas of absence of the surface epithelium (*a*) opposite to which there is a many-layered cylindrical epithelium with a suggestion of tufting and papillary formation and hydropic cells (*b*). Leucocytic infiltration of the mucosa.

where stratified cylindrical epithelium, often showing a tufted and papillary formation. The mucosa in certain portions is desquamated so that peculiar cell groups are found in the lumen; these contain hydropic cells without visible cell borders. In certain areas the surface epithelium

may be entirely missing while at the same time the contiguous site may show a stratified cylindrical epithelium with papillae and hydropic cells.

So far we have shown that the uterine mucosa after chronic treatment with follicle hormone shows typical changes, to wit: localized

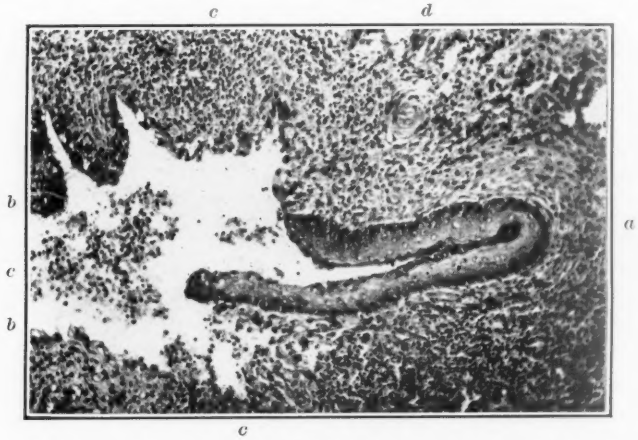

Fig. 3.—100 magnifications. Partial metaplasia of the uterine mucosa (*a*). One of the areas which is not metaplastically changed, shows only a single layer of epithelium (*b*). A massive leucocytic migration into the lumen (*c*). Gland lined with cylindrical epithelium (*d*).

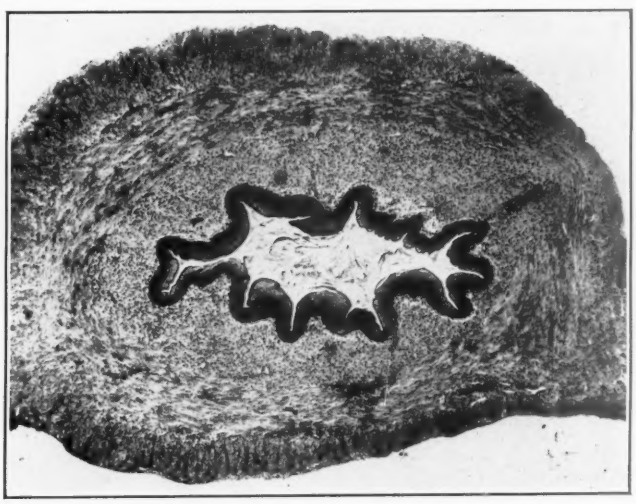

Fig. 4.—Cross-section of the uterus, 36 magnifications. Total metaplasia of the mucosa with stratified squamous keratinized epithelium, the uterus having the appearance of a vagina in estrus.

absence of epithelium, next to normal epithelium, as well as stratified cylindrical epithelium and papillary projections into the lumen of edematous high cylindrical cells, subepithelial leucocytic infiltration (eosinophiles) with transmigration into the lumen. It is of particu-

lar interest that the uterine epithelium can undergo a complete metaplasia under the influence of follicle hormone so that at first glance the uterus may appear like the vagina in estrus. Fig. 3 shows a partial metaplasia of the uterine mucosa in which we see leucocytes in the lumen and a metaplastic mucosa protruding into the lumen. The leuco-

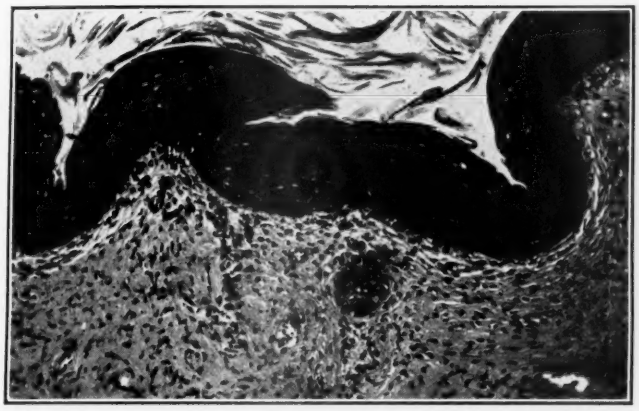

Fig. 5.—Same as Fig. 4, magnified 150 times. Oil immersion. Section of the totally metaplastic uterine mucosa. Horny lamellae extruded into the lumen. In addition there is a metaplasia of a gland lining (*a*).

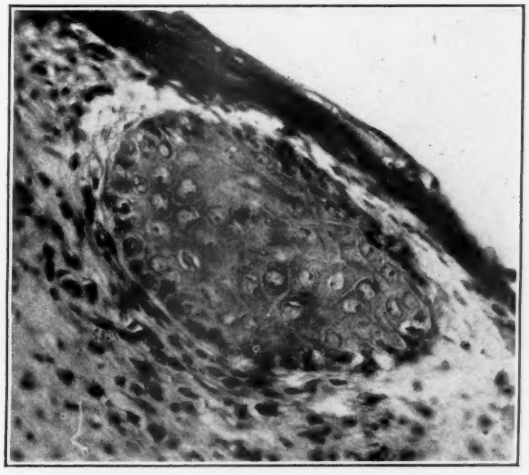

Fig. 6.—400 magnifications. Oil immersion. Metaplasia of uterine mucosa. Flattened horny stratified epithelium. In the mucosa a gland completely filled with metaplastic stratified epithelium showing prickle cells and intercellular bridges.

cytes reach the horny layer. That portion of the mucosa which is not metaplastic shows a single layer (Fig. 3, *b*) of epithelium not the normal cylindrical. In the stroma there is a gland lined by squamous rather than cylindrical cells (Fig. 3, *d*). The whole mucosa shows a thick leucocytic infiltration predominantly eosinophilic, as well as a perivascular infiltration of the muscularis. Fig. 4 shows a complete meta-

plasia of the uterine mucosa, the lumen lined by a stratified keratinized squamous epithelium, so that the uterus resembles a vagina in estrus. As in the case of the estral vagina, horny lamellae are shed into the lumen, the uterine cavity here is filled with them. The higher magnification of Fig. 5 shows this plainly. Here we also see a gland in the depth of the mucosa metaplastically altered into squamous epithelium, with prickle cells and intercellular bridges, the latter most plainly seen in Fig. 6, from another experiment. In Fig. 6 we note as well the metaplasia of the mucosa with flattened keratinized squamous epithelium. Just under the mucosa there is a gland lined by metaplastic

Fig. 7.—Cross-section of a uterus, 150 magnifications. Mucosa varies in composition. At (*a*) stratified cylindrical epithelium. At (*b*) papillary projections into the lumen of edematous high cylindrical cells. At (*c*) complete absence of epithelium. At this point a polyp of uncornified stratified epithelium projects into the lumen (*d*). Subepithelial gland lumen filled with metaplastic stratified epithelium.

squamous epithelium in which the prickle cells and intercellular bridges can be recognized. The last stage is the complete suppuration of the uterus, changing it into a sac the thickness of a thumb, filled with pus (Fig. 9). The cavity of the uterus is enormously dilated (Fig. 8) contains desquamated horny lamellae, cell detritus, and pus. The mucosa of the uterus shows a circular purulent liquefaction and contains numerous abscesses. In part the continuity is interrupted and is held together by the serosa alone. Those portions of the muscularis which have fallen victim to the suppuration show heaps of pseudoxanthoma

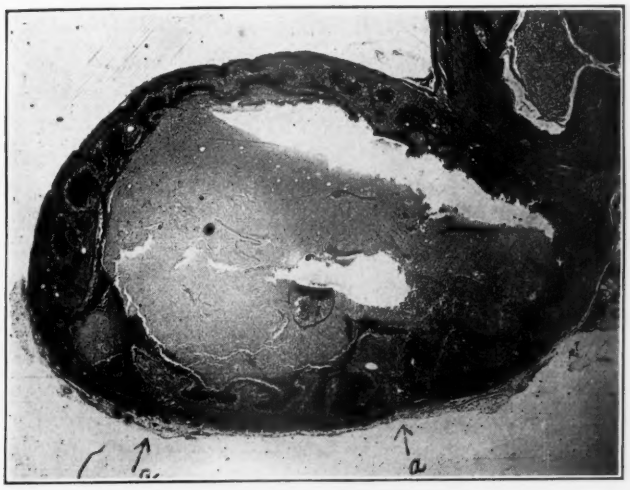

Fig. 8.—Cross-section through the uterus, 9 magnifications. Uterine cavity widely dilated, in the lumen there is pus and detritus. Uterine wall is studded with abscesses some of which reach the serosa. At various points, heaps of pseudoxanthoma cells (*a*).

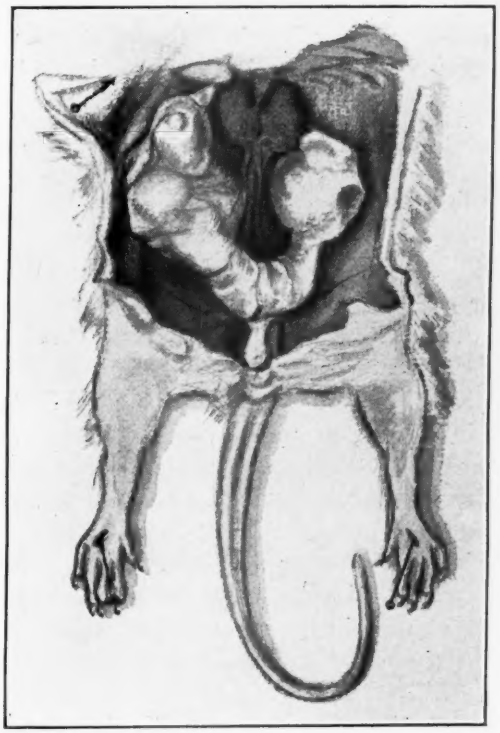

Fig. 9.—Uterus of a rat after eight months' treatment with estrogenic hormone (dimenformon). Both horns converted into huge pus sacs.

cells (Fig. 8, *a*), which determine the macroscopic yellow color of the uterine horns. We are evidently dealing with a long-standing process. We were able to observe the suppuration clinically in various stages and it could be diagnosed by the appearance of pus in the vagina. In some cases the uterus seemed barely enlarged (the thickness of a slate pencil), nevertheless on section creamy pus was found in the lumen. In other cases the pus sacs were tremendous as, for instance, in Fig. 9. Some of the animals died of peritonitis. Bacteriologic investigation of the uterine secretion showed gram-positive diplococci and bacilli, gram-negative bacilli, and occasional fusiform bacilli.

In brief, then, it is evident that as a result of chronic treatment with follicle hormone the following changes are seen in the uterus of the rat: multilayered cylindrical epithelium with polypoid formation, focal absence of the surface epithelium, focal desquamation of areas of mucosa with hydropic cells without visible cell boundaries, partial to total metaplasia of the uterine mucosa with discharge of horny lamellae into the lumen, metaplastic alteration of the glands, stratified epithelium, leucocytic infiltration of the mucosa with transmigration of leucocytes into the uterine cavity, pyometra with abscess formation of the musculature, and consequent exitus.

In an earlier study[3] I have described the effect of chronic treatment with follicle hormone on the uteri of rabbits in which four characteristic reactions were determined:

A. Hyperemia of the endometrium and musculature and occasional small mucosal hemorrhages
B. Glandular cystic hyperplasia of the endometrium
C. Infarctlike necrosis of the endometrium
D. Aseptic suppuration of the uterine cavity

The glandular cystic hyperplasia is caused by a direct action of follicle hormone on the endometrium. The other reactions are the result of thromboses. There is a consequent infarctlike necrosis and secondary to this, an aseptic suppuration of the uterine cavity. In the case of the rat the leucocytic infiltration appears very early so that it is not surprising that there is ultimately a partial infection, complete suppuration and disintegration of the uterus. I have not noted metaplastic changes of the mucosa and of the glands in rabbits nor have I seen thromboses and infarctlike necrosis in rats. Hence, we can deduce that the uteri of rats and rabbits react differently to treatment with follicle hormone. When I stated in the above-quoted study that chronic treatment with follicle hormone evoked no determinable changes in the uterus, I was wrong. I had treated rats and rabbits for the same length of time and saw no effect on the rats, but by continuing the treatment of the rats one can obtain the reaction that I have described today.

Selye, Thomson and Collip[4] report that they have obtained a beginning metaplasia of the uterine mucosa in three cases and a complete

metaplasia in one case by treating castrated rats with follicle hormone after previous ligation of the uterine horns. We have here the combined action of a mechanical irritant and a hormonal one, reminding us of the well-known researches on experimental placentomas (Gloeb, Calderini, Long and Evans and others). In my own investigations, on the contrary, we are dealing with effects produced by hormones alone.

After my work was completed there appeared the publications of Gumbrecht[5] and Migliavacca[6] who also describe metaplastic changes in the mucosa as a result of long-continued treatment with follicle hormones. Migliavacca in a very interesting study shows that plugs of squamous epithelium project deep into the wall of the uterus up to the smooth musculature. He describes, in addition, considerable invasion of the myometrium by these projections and discusses the question of a precancerous lesion. From my own investigations I have never had the impression that we were dealing with a process which could in any sense be designated as malignant. We cannot conclude from the above reactions that malignant tumor formation can be produced by treatment with follicle hormone. Although Migliavacca used some non-castrated animals, most of his experiments were on castrated animals. After castration prolan A is produced in increased quantity by the pituitary, so that castrated animals in whom one injects follicle hormone over a long period are under the influence of two hormones, both prolan A and follicle hormone. Perhaps this plays a rôle in the experiments of Migliavacca. In any event I should like to emphasize that even after seven months of treatment of rats with enormous doses of estrogenic hormone (1,080,000 M.U. dimenformon) I have never seen pictures which one could interpret as malignant tumors or precancerous lesions, if such exist. It may be mentioned that Migliavacca in a previous study[7] has described changes in the mucosa such as reduplication of layers, vacuolization, hydropic degeneration as a result of long-continued treatment with follicle hormone.

CONCLUSIONS

The effect of long-continued treatment with follicle hormone on the uterus in the rat is described. Though the inhibitory effect upon the anterior lobe of the pituitary by follicle hormone, expressed in eunuchoid dwarfism, appears uniformly in all the animals, the local effect on the uterus itself varies widely. Sometimes there is no effect, sometimes a complete destruction of the uterus. The effect of the estrogenic hormone therefore varies individually. The following characteristic effects were established:

1. Effects on the Epithelium.—A. Marked variability, focal absence of the epithelium, stratified cylindrical epithelium, tufts and papillae projecting into the lumen, desquamation of whole areas of the mucosa, with hydropic cells without visible cell boundaries.

B. Partial to total metaplasia of the surface epithelium into stratified keratinized squamous epithelium, the uterus having the appearance of a vagina in estrus.

2. *Metaplasia of the glands*, change of the normal glandular epithelium into squamous epithelium with prickle cells and intercellular bridges.

3. *Inflammatory changes*, leucocytic infiltration of the entire mucosa, a subepithelial wall of eosinophiles, transmigration of leucocytes into the uterine cavity, suppuration of the mucosa (pyometra) and destruction of the musculature with conversion of the uterine horns into pus sacs of the thickness of a thumb.

Suppuration of the uterus also occurs in rabbits as a result of long-continued treatment with follicle hormone. This suppuration is aseptic and occurs after thrombosis and resultant necrosis. In contrast to this there is a secondary infection in the case of the rat. The ovaries of the experimental animals show a high grade atrophy, occasional enlarged follicle, never a corpus luteum.

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NOTES FROM A PREGNANCY DIAGNOSIS LABORATORY (1936)

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THE state, concerned with the problems that must arise out of the dwindling of a population, a change in the mean age, and a swing in the sex ratio, is demanding knowledge of the biologic as well as of the economic factors that influence the wish and the ability of individuals to reproduce. Society demands that medicine shall gain control of those wasteful derangements peculiar to women, and shall rid childbearing of baseless dread and preventable danger. Medicine is responding. The search for new knowledge concerning human sex and reproductive physiology is eager, and the application of biologic science to human affairs follows immediately upon discovery. It is in this field of enquiry that intellectual adventure is to be most readily encountered, and that kind of immortality which a scientist hopes to achieve is to be earned. Among those whose names will certainly be respectfully and gratefully remembered in the history of this branch of medicine is that of the man to whom this volume is a memorial. We who write have shared his joy in his discoveries, and we gladly pay tribute to the quality of the man and of his work. To him this Laboratory is greatly and continually indebted; upon his knowledge and wisdom it has frequently relied.

Among the instruments of precision that medicine has devised and used in the execution of its purposes and ideals, there is none finer than the biologic test for early pregnancy. Its value to the profession, to the general public, and to the state is amply illustrated in this Report of a Pregnancy Diagnosis Laboratory which was organized some six years ago in order to place the then-new exploitation of scientific discovery at the service of the medical profession of Great Britain. It was thought then (and nothing has occurred since to disturb this view) that a specialized laboratory with its own breeding plant, its duplicated and highly-skilled staff, and operating on a large scale, could best give an adequate service with graded fees that would make the tests available for all, irrespective of income category, and, at the same time, accumulate data sufficient for profitable analysis.

In 1936, 7,193 specimens were examined. Of these, 3,791 fell into the "full-fee" class, while 3,402 came from patients in poor circumstances who were asked to pay according to their means.

The tests were distributed as shown in Table I.

The fact that so many more Aschheim-Zondek tests than Friedman tests are carried out demands an explanation. The Friedman test must necessarily be more expensive than the Aschheim-Zondek; rabbits are more difficult to breed, to procure, and to accommodate in large numbers

TABLE I

			POSITIVE	NEGATIVE	UNFINISHED	DISAGREEMENT	CONFIRMED AS CORRECT
For ordinary pregnancy diagnosis	Aschheim-Zondek	6537	3415	3092	30	--	2001
	Friedman and confirm. A-Z.	388	197	173	1	23	201
	Friedman alone	83	42	38	3	--	33
		7008	3654	3303	34	17	2235

	MODIFIED A-Z
For detection of hydatidiform mole, chorionepithelioma, or recurrence of these	171
For detection of malignant tumor of the testis in the male	14
For quantitative estimation of sex hormones	13

than are immature mice; the adult rabbits which are used in this Laboratory require to be isolated for at least four and a half weeks before use; an injection into a rabbit's ear-vein requires more skill than does a subcutaneous injection in the mouse; and, finally, it is very difficult in our experience to purchase rabbits free from disease. It would appear, moreover, that in the majority of cases in which the help of this Laboratory is sought, urgency is not a serious consideration: a report after five days is quite sufficient. Analysis of the records shows that the Friedman test is required mainly in those cases in which the size of the fee has not to be considered; in which urgency exists: e.g. in the differential diagnosis of ectopic gestation; in cases in which, if pregnancy exists, it must be terminated for medical reasons; and in cases in which the information sought is urgently desired for the reason that, if pregnancy is shown to exist, certain domestic arrangements must be disturbed. The Laboratory offers a choice of tests; it is the practitioner who decides. The great preponderance of the Aschheim-Zondek in our records has always been a matter of surprise to me, for I had assumed that the rapid test would be the one in greater demand. However, I am very content that the demand is mainly for the slower Aschheim-Zondek, for though the Friedman in our hands is undoubtedly the more delicate of the two, the Aschheim-Zondek is the more convenient procedure when very many tests are being carried out every day, and moreover, the graded reactions that are obtained therein provide the means of distinguishing between several relative concentrations of the gonadotropic hormones in different specimens, and permit us to refer a strong positive, a rather weak positive, a weak positive, an extremely weak positive, a negative with uterine and

vaginal enlargement to a variety of different conditions. Thus, a weak positive following upon a sucession of strong positives in a case of habitual abortion permits us to warn the doctor that all may not be well. A weak positive in a case in which abortion is suspected to have occurred provides support for this diagnosis. A negative with vaginal and uterine enlargement at once raises the question as to the age of the patient.

The Friedman test is always used in cases in which medicolegal interest is concerned and is followed up by an Aschheim-Zondek. In such cases a preliminary laparotomy is performed on two rabbits, and their ovaries are examined to ensure that ovulation has not recently occurred. Ten cubic centimeters of filtered urine is then injected into the ear-vein of each. Thirty hours later one rabbit is killed. If it shows a positive result, the second rabbit is returned to store, if negative, a further 10 c.c. is injected into the second rabbit, and this animal is killed thirty hours later. If this one also gives a negative, this result is regarded as trustworthy.

The combined Friedman and Aschheim-Zondek is the test that is to be preferred in the case of suspected ectopic gestation. The information that these tests give in such cases has proved time after time to be of the greatest possible value.

Specimens from a series of cases of ectopic gestation operated upon in the Royal Infirmary of Edinburgh were examined. Specimens from 14 cases taken within eight hours after the operation, and thereafter daily for seven days, were tested, and in every case a definite negative test was invariably obtained seventy-two hours after the removal of the embryo. To the surgical and nursing staff of the Wards concerned, I wish to offer my thanks.

In a very considerable number of cases, the question of fetal death was raised. In these cases the tests have not proved to be entirely satisfactory. It is true that commonly the "strength" of the reactions on the part of rabbit and mouse have been such as to indicate a relatively low concentration of gonadotropic hormones and thus support the tentative diagnosis. Indeed, definite negatives are not uncommon. But in other cases quite strong positives have been obtained as long as a month after the time when, according to the clinical evidence, the fetus had perished. However, for the present, the combined Friedman and Aschheim-Zondek is the most useful test in these cases. The quantitative blood estrin test does not appeal to the general practitioner, for the obvious reason that it is far easier to obtain a specimen of urine than one of blood.

ERRORS

In the Aschheim-Zondek Tests: 16 false negatives, and 5 false positives.

Three of these false negatives are to be explained by the fact that conception had too recently taken place. In one case it was known that

conception had occurred twelve days, and in another fourteen days, before the specimen was taken. In yet another, in which it was known that pregnancy was of very short duration, if it existed at all, the test, repeated ten days later, gave a positive reaction. Experience has shown that too much reliance cannot be placed upon a negative Aschheim-Zondek result in the case of a patient less than one month pregnant. In 11 of the remaining cases the duration of pregnancy had been longer, four weeks in 1 case, seven weeks in 1, eight weeks in 2, fourteen weeks in 2, sixteen weeks in 1, nineteen weeks in 1, and twenty-four weeks in 3. One false negative was obtained in a six weeks' pregnancy that ended in abortion, and another related to a case of pregnancy in a woman suffering from Graves' disease. This case was of particular interest, and the patient was followed throughout pregnancy. At the second month the Aschheim-Zondek test was negative, at the fourth month positive, at the fifth month negative, and at the seventh positive (weak) again.

Of the five false positives, two are without any explanation. The doctor in charge of one case, on clinical grounds, had reason to doubt the correctness of this result. The test was repeated one month later to give a straight forward negative test. In the other, the patient had left the district and nothing was known of her subsequent history. In the remaining three cases, a weak positive result was recorded (faint blood spots and white spots in two mice only). In one of these the patient was suffering from "congestive heart disease"; in another, she had been receiving treatment for "thyroid and pituitary disturbance," and the third, suffering from hemorrhage, had been curetted.

In addition to these errors, a number of unclear results were obtained in the case of specimens derived from single women greatly fearing pregnancy. Two factors undoubtedly affected the value of the reports issued in these cases. In the first place it seems clear that the emotional condition of the patient can have endocrine repercussions which can affect the reaction of the mice, and second, in the case of patients by whom pregnancy is feared, the incorrectness of a positive result strongly suggests that abortion has taken place. When the history supports this explanation, the result is not regarded as incorrect.

As in previous years, the errors are found to be concentrated at the beginning and at the end of the reproductive phase of life. The young unmarried girl aged thirteen to seventeen years, and the woman of forty-three plus, are very unsatisfactory in respect of these biologic tests for early pregnancy.

NEGATIVE ASCHHEIM-ZONDEK RESULTS—REPETITION ADVISED

In no less than 573 cases in which a negative Aschheim-Zondek result was recorded, it was necessary (for the reason that though the ovaries of the mice remained unaffected, their uteri and vaginae were much enlarged) to send with the report a slip intimating that though according

to the standards used this result must be returned as a negative, yet the vaginal and uterine enlargement indicated that in the specimen the concentration of hormones was unusually high and that this might mean one of several things: e.g. very early pregnancy, early pregnancy in a woman with exceptionally low hormone concentration, menopausal state, incipient menstruation (after a phase of amenorrhea), fairly recent death of the ovum, lactation, or some general endocrine disturbance not connected with pregnancy. (Experience has shown, also, that this uterine and vaginal enlargement is to be expected and disregarded in mice that are too large or too old and which should not have been used in these tests.) In these cases the practitioner is advised to have the test repeated in about ten days' time if doubt persists.

Of the conditions which are known to be associated with this uterine enlargement, early pregnancy, of course, is the only one which, if the test were repeated, would contradict the negative result first obtained. Of the 573 cases in which this slip was attached to the report, no confirmation was received in respect of 406. In 151 the negative result was found to be in harmony with the subsequent clinical history, while in 16 pregnancy had existed at the time of the test. For the reason that, in the reports issued in these cases, it was stated that early pregnancy could not be excluded, these results are not regarded as errors. Though the practitioner who receives such a result is naturally inclined to be irritated, for he has such implicit faith in these tests as to demand a plain unqualified positive or negative, it is our practice to furnish him with a full and complete statement of the result obtained. It is for him to make the diagnosis. The report issued from this Laboratory is but one of many factors that he must evaluate in arriving at his diagnosis.

A review of the records of all the Aschheim-Zondek tests carried out here during the last six years makes it quite clear that the concentration of gonadotropic hormones in different women during early pregnancy varies very widely. Very rarely is it such as to leave the mice completely unaffected when a false negative is obtained. Less rarely is it so low as to produce only uterine and vaginal enlargement, and without a knowledge of the age and history of the patient, all that can be done is to recommend that the test be repeated later.

In 23 cases the Friedman and Aschheim-Zondek tests disagreed. These disagreements provoke much anxiety and dissatisfaction. The disagreement between the Friedman + and + (weak) and the Aschheim-Zondek – and – (slip) is more apparent than real. Under the conditions that obtain here the rabbit picks up a concentration of gonadotropic hormones to give a +, while in the Aschheim-Zondek a – or – (slip) is obtained. This is the result that is to be expected in very early pregnancy, in pregnancy of four or six months' duration, in cases in which the hormone concentration is unusually low and in cases of recent fetal death. The

same result [Friedman + (weak) and Aschheim-Zondek – (slip)] may be yielded by the girl around the time of puberty in cases of rape and assault, and in these a negative is likely to be the correct interpretation. It is more difficult to explain away the Friedman negative and Aschheim-Zondek positive disagreement. If one must choose between the + and the –, then it is safe, as a rule, to prefer the +. Refractory rabbits are not uncommon. But the + is not always correct as is seen in the first confirmation.

TABLE II. DISAGREEMENT BETWEEN FRIEDMAN AND ASCHHEIM-ZONDEK TESTS

NO. OF CASES	FRIEDMAN	ASCHHEIM-ZONDEK	CONFIRMATIONS
5	–	+	1. Friedman – correct, yet every one of the 5 mice in the A-Z gave a strong +. Single woman greatly fearing pregnancy. Last menstrual period 6 weeks ago. 2. A-Z + correct. Thought to be a case of ectopic gestation. Operation revealed uterine pregnancy. Fetus alive at time of test. Died later when A-Z repeated gave –. 3. A-Z + correct. 4. A-Z + correct. Patient 1-2 months pregnant at time of test.
4	+	–	1. A-Z – correct. Friedman repeated 1 week later gave –. 2. A-Z – correct. Operation disclosed fibroid. Friedman was rather indefinite +, a – report issued.
11	+	–(slip)	1. Friedman + correct. Patient 3 weeks pregnant at time of test.
3	+(weak)	–(slip)	1. A-Z – correct (Friedman first rabbit –?; second +?). Young single girl. 2. A-Z – correct. Repeated later gave +. Medico-legal case of girl aged 15½, 4½ months pregnant at time of test. 3. Case of incomplete abortion with greatly degenerated decidual tissue and a dermoid in the left ovary.

TOXIC SPECIMENS

In thirty-four cases the test was not completed for the reason that the injections killed the animals concerned. In two of these the urine on receipt was of a bright green color, and enquiry elicited the fact that brilliant green had been painted upon the patient's cervix some time before the specimen was taken. All attempts to remove this from the specimen failed, and the injections were almost immediately lethal. As has been noticed frequently, many of the specimens that fall into this uncompleted class are derived from single women who have reason to wish to avoid pregnancy. It would seem that quite commonly some attempted chemical interference with pregnancy is the reason for the lethal properties of the specimen.

DILUTION TESTS

In those cases in which hydatidiform mole or chorionepithelioma is suspected, in addition to the ordinary Aschheim-Zondek, in which prepared undiluted urine is used, additional groups of mice are injected with urine diluted 9 and 99 times its own volume with distilled water. Experience has shown that while normal pregnancy can give a positive with undiluted urine and with the 1/10 dilution, it is exceedingly rare for it to do so with the 1/100; in fact, so rare that a positive 1/100 is regarded as being strongly indicative of mole or chorionepithelioma. In cases in which the mole has been expelled or removed, these tests are repeated at monthly, and later at bimonthly, intervals in order that recurrence may be recognized. There were 171 such dilution tests carried out during the year. It is the rule that a hydatidiform mole or chorionepithelioma gives +++, that after removal there is a gradual and progressive swing toward ---. This result was obtained in some cases as early as one week after the evacuation, but this is rare; usually this result is obtained about three months afterward. In one case a +--, in the absence of recurrence, was obtained as late as one year after removal.

Urines from 14 males were examined by these dilution tests in cases in which malignant diseases of the testis were suspected or in which a malignant tumor had been removed.

Urine from treated cases of seminoma persistently gave negative results. In two cases only were positive results obtained. In one +, - (slip), - (slip), orchidectomy revealed an embryonal-celled carcinoma; in the other + (weak), -, -, a carcinoma of the testis was found on operation.

GENERAL

In addition to the cases of particular interest so far mentioned, it is perhaps advisable to record that negatives, later confirmed, were obtained in cases of tuberculous peritonitis, large cyst in the broad ligament, parovarian cyst, chronic salpingitis, torsion of the fallopian tube, endometrioma of left ovary, and following the removal of a luteinoma of the ovary, as well as in several instances of carneous mole and of pseudocyesis. Positives, later confirmed, were obtained in a case of ovulation without menstruation, in another in which there was very severe hydramnios in a mental patient previously sterilized, and in others in which deep x-ray therapy had previously been exhibited. In one case in which mole was suspected because of the disproportionate size of the uterus, a +-- was obtained in the dilution test, and the final diagnosis was twins. Positives were also obtained in cases in which patients had been married for twelve, fifteen, and sixteen years, respectively, without having produced any issue during this time. On the other hand, a positive was also obtained in the case of a woman who had been married exactly twenty days prior to the test. In two cases in which, following

x-ray examination, pregnancy had been stated not to exist, positives, which were later confirmed, were obtained.

One point that may be of interest to others who are carrying out these tests is that about 1 in every 100 packages containing specimens is broken in the post. In order to cope with this misfortune, a small hand press was devised. Into this, the cotton wool, cardboard, and correspondence is placed and the fluid expressed therefrom. Such treatment in no way affects the properties of the specimen.

TESTS IN RELATION TO THE AGE OF THE PATIENT

The practitioner is requested, when sending in a specimen, to state the age of the patient, the date of the last menstrual period, and any other detail of the clinical history that he thinks might be of help in the interpretation of the result obtained. In 2,375 cases the age of the patient was given. It ranged from thirteen to sixty-five over the following distribution:

AGE	13	14	15	16	17	18	19	20	21	22	23	24	25
NO. OF TESTS	2	8	11	21	24	29	40	53	78	75	77	98	107
AGE	26	27	28	29	30	31	32	33	34	35	36	37	38
NO. OF TESTS	103	116	96	29	115	79	112	77	85	97	83	60	63
AGE	39	40	41	42	43	44	45	46	47	48	49	50	51
NO. OF TESTS	63	95	59	72	58	48	50	41	21	19	18	10	6
AGE	52	54	65										
NO. OF TESTS	4	1	1										

This range covers the whole of the ordinary reproductive phase of the individual life with slight extensions at both ends. The number of tests rises sharply with the age of the subject to reach a peak at 24 to 27. The fewness of the specimens from women of twenty-nine years seems to require some explanation. From a second peak at 32 the number falls more or less gently to the end.

Of the two cases in the thirteen age group, one was positive and was of interest in that the doctor reported that the hymen remained imperforate. Positives were given by two of the girls of fourteen; 5 of those of fifteen; 3 of the sixteen class, together with 1 which turned out to be a hydatidiform mole; 10 of those aged seventeen. At the other end of the scale, every one of the fifty age class gave a negative result; of the fifty-one, one was a mole and the rest negative; of the fifty-two, one was a mole, one positive and one negative. The woman aged fifty-four years was negative, as was also the woman of sixty-five who was shown to have a very large ovarian cyst.

DISTRIBUTION OF TESTS IN RELATION TO THE DATE OF THE LAST MENSTRUAL PERIOD

In 2,049 cases the date of the last menstrual period was recorded among the particulars sent with the specimen. It will be seen that in 97 cases no menstrual period had yet been missed. These are the cases

in which, commonly, medicolegal interests are involved or in which the earliest possible diagnosis of pregnancy following exposure is required for the reason that therapeutic abortion has to be considered. In a considerable number of these tests, the question of ectopic gestation had arisen. But undoubtedly the number includes a great many which can

TABLE III

Weeks after last menstrual period	1	2	3	4	5	6	7	8
No. of cases	12	22	21	42	266	319	169	373
Positive	2	9	9	15	107	174	98	224
Confirmed	0	3	4	5	41	51	23	71
Negative	10	13	12	27	109	145	71	149
Confirmed	4	3	5	13	39	43	20	38
Errors	–	2	–	1	–	–	1	2

Weeks after last menstrual period	9	10	11	12	13	14	15	16
No. of cases	146	105	57	225	31	35	10	86
Positive	107	69	33	128	17	16	8	54
Confirmed	35	17	10	38	2	2	1	15
Negative	39	36	24	97	14	19	2	32
Confirmed	12	8	10	26	4	4	1	8
Errors	–	–	–	–	–	2	–	1

Weeks after last menstrual period	17	18	19	20	21	22	23	24
No. of cases	14	26	3	47	3	9	0	34
Positive	8	16	0	27	2	7	0	13
Confirmed	1	5	0	6	1	3	0	7
Negative	6	10	3	20	1	2	0	21
Confirmed	2	3	0	5	1	0	0	3
Errors	–	–	1	–	–	–	–	3

Weeks after last menstrual period	26	28	29	30	32	33	34	35	36	40	44	48	52	104
No. of cases	1	19	1	1	7	2	2	1	1	2	1	1	4	1
Positive	0	11	0	0	0	2	0	0	1	0	0	1	2	0
Confirmed	0	4	0	0	0	0	0	0	0	0	0	0	0	0
Negative	1	8	1	1	7	0	2	1	0	2	1	0	2	1
Confirmed	0	2	0	1	1	0	1	0	0	0	0	0	1	1
Errors	–	–	–	–	–	–	–	–	–	–	–	–	–	–

only be regarded as testimony to the doctor's faith in the almost magical efficacy of these tests. In two cases it was actually discovered on enquiry that fertilization, had it occurred, had taken place no more than twelve hours previously. Negative results are almost twice as frequent as are positives in specimens taken up to four weeks following the last menstrual period. This is as would be expected. It is indeed somewhat surprising that only 3 of the errors fall into this group.

The cases in which one period only had been missed (i.e., five to eight weeks after the last menstrual period) are the most numerous, constituting no less than 1,127 out of 2049 cases. This is quite as it should be, for it is here that the pregnancy diagnosis tests should and do provide the greatest help. In this group the positives outnumber the negatives, 603 : 474, and only 3 errors require to be recorded.

From this point onward the reason for the test would seem to change somewhat. It becomes more and more a matter of differential diagnosis and less a diagnosis of pregnancy before the clinical picture becomes clear. Fetal death is a much more frequent cause, owing to the fact that the enlargement of the uterus has fallen out of step with

TABLE IV. QUANTITATIVE ESTIMATIONS

DOCTORS' DIAGNOSIS AND DEMANDS	ESTRIN INTERNATIONAL UNITS PER LITER	GONADOTROPIC HORMONE RAT UNITS PER LITER	MALE HORMONE INTERNATIONAL UNITS PER LITER	
1. Fluid from breast cyst. Estrin estimation	30 in the 12 c.c.	-	-	Freed's method for blood because of large amount of protein contained in the fluid
2. Granulosa celled tumor of left ovary. 10 c.c. of urine taken just before operation	= 50			
30 c.c. taken 24 hr. after operation. Estrin estimation	= 33			
3. Endocrine disturbance, obesity, amenorrhea, milky secretion in breasts and bluing of vulva. Estrin and gonadotropic estimation	12	> 1		Aschheim-Zondek
4. Myxedema. Estrin and gonadotropic estimation	> 3	> 1		
5. Estrin deficiency. Estrin estimation	10			
6. Persistent amenorrhea. Estrin and gonadotropic estimation	2	12		
7. Amenorrhea. Girl of 15. Blood estrin	app. 2 in 40 c.c.			
Urine estrin	8			
8. Irregular menstruation. Estrin and gonadotropic estimation	12	2.5		
9. ? Estrin and gonadotropic estimation	10	< 1		
10. ? Estrin and gonadotropic estimation	11	2.3		
11. Amenorrhea with hirsutes. Male hormone			36	No free ♂ hormone
12. Hermaphrodite. Estrin and male hormone	30		120	
13. Seminoma testis (postoperative). Male hormone estimation		< 1		

the duration of pregnancy. The positive results continue to outnumber the negatives until the last two groups are reached, and, as would be expected, in the last group (26 to 104 weeks after the last menstrual period) the negatives greatly preponderate.

QUANTITATIVE ESTIMATIONS

During the latter part of the year, in response to demands made upon the Laboratory, arrangements were completed whereby quantitative estimations of estrin, gonadotropic and male hormones in blood and urine could be undertaken. Though in my view it is as yet doubtful that the information that this Laboratory can supply can be of any considerable value to the clinician, certainly anything that can aid in the advancement of our knowledge concerning these hormones must eagerly be undertaken in order that the time may come when endocrine therapy may be securely based upon a sufficient body of accurate knowledge. At the moment the results of these estimations possess an interest which is mainly academic. For the extraction from the urine of estrin and male hormone a modification of the Gallagher and Koch method is used; for the gonadotropic hormone the alcohol precipitation method is employed; for blood estrin and gonadotropic extraction Freed's method is used. Groups of ovariectomized mice are used in the assays of estrin; capons for the male hormone; and immature female rats for the gonadotropic hormones. The amounts of estrin and male hormone are given in international units per liter; that of gonadotropic hormones in rats units per liter, the rat unit being that amount required to produce a 33 mg. ovary in an immature female rat of 40 gm. body weight under the conditions that obtain here.

ON THE INACTIVATION OF ESTRONE, ESTRADIOL, AND THEIR MONOBENZOATES IN THE ORGANISM

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IN ORDER to understand the mechanism of the action of estrogenic compounds, especially as to their therapeutic effects in human subjects, it is of considerable importance to know the fate of the estrogenic substances in the body. Our investigations on this subject consist of experiments with human beings as well as with animals. Those carried out with human subjects will be published elsewhere; in this paper, only the results obtained from experiments with animals will be recorded.

PREVIOUS INVESTIGATIONS

Some years ago Frank, Goldberger, and Spielman (1932) observed that half hourly blood samples taken from isolated adult rabbits, into which 2,000 M.U. of estrogenic hormone were injected intravenously in one dose, yielded only 1 M.U. in 4 c.c. of serum during the first hour and less thereafter. A rabbit injected with 3,000 M.U. and killed twenty-four hours later by bleeding showed less than 2 M.U. in the total blood volume. Fee, Marrian and Parkes (1929) using the heart-lung-kidney preparation, after adding 200 "mouse units" of estrone to about 500 c.c. of blood in circulation, could detect estrone in none of the organs perfused in this preparation. These authors think it probable that the hormone is destroyed by oxidation while passing through the lungs.

In an extensive investigation on the fate of estrogenic substances in the animal organism, Zondek (1934) made the following observations: After administering estrone subcutaneously to immature rats in amounts up to 20,000 M.U., no hormone at all was excreted in the urine. If amounts up to 40,000 M.U. of estrone were injected subcutaneously into immature rats and the animals were killed at different intervals (three to seventy-two hours) afterward, no more than 2 per cent could be recovered after neutral and 20 per cent after acid extraction. A possible conversion of estrone into estriol could not be proved.

When, however, immature rats received estrone benzoate injected subcutaneously, forty-eight hours after the injection, 80 to 90 per cent of the amount injected could be recovered from the extraction of the entire rat. According to Zondek this phenomenon explains Butenandt's (1930) finding of the protracted estrogenic action of estrone benzoate and confirms his view that the action of benzoate is due to its being slowly hydrolyzed in the body. From the fact that estrone benzoate is still active in the organism under circumstances in which estrone is not, this author discusses the possibility that the inactivating process starts at the phenolic hydroxyl group of the estrone molecule which in estrone benzoate is protected by the esterification with benzoic acid. From in vitro experiments, he ascertained that the liver probably plays a part in the inactivation; for, after shaking estrone with mashed liver of immature rats at 37° C., a strong inactivating effect could be observed in most cases. This effect was not obtained when the mashed liver was heated beforehand to 70° C.

Our investigations were carried out with pure estrone* and estradiol; and with the benzoic ester of estrone and the monobenzoic ester of estradiol. All crystalline hormones were injected subcutaneously, dissolved in 1 c.c. of olive oil. As test animals we used mature female rats weighing between 150 and 200 gm. At the end of the experiment these animals were killed by a blow on the head.

A. EXCRETION OF ESTRONE IN URINE AND IN FECES OF MATURE FEMALE RATS AFTER SUBCUTANEOUS ESTRONE ADMINISTRATION

In the first experiments the excretion of estrone in the urine and feces of rats was investigated after injection of relatively large quantities of the hormone. Two test animals received subcutaneously 25,000 R. U. dissolved in oil. The urine and feces were collected during four days after the injection and worked up separately. The urine, including the wash water with which the cage had been rinsed, was refluxed with benzene on a water-bath for three periods of four hours each, the benzene being renewed each time. To each liter of residual water-urine mixture, 150 c.c. of 25 per cent hydrochloric acid were added. The acid mixture was extracted with benzene three times. The acid benzene extracts were united and washed with water. Both the neutral and acid benzene extracts were then evaporated and the residues dissolved in oil and tested for their estrogenic hormone content in ovariectomized mice (de Jongh and others, 1932). The neutral extract proved to contain 150 and 50 estrogenic units, respectively, while in the acid extracts in both cases, less than 50 estrogenic units could be detected. In the urine collected during four succeeding days after the injection, the maximum found was 150 units, that is 0.6 per cent of the amount of estrone injected.

The feces proved to contain 100 and 750 units, respectively; extraction was carried out in the acid medium in the manner described above. Although not more than 3 per cent of the injected amount could be extracted from the feces, this is still more estrogenic substance than is excreted in the urine. After this, the urine and feces were collected during another four days. In only one case could more than 50 M.U. be detected in the urine or feces. (In the urine of the second test animal 100 M.U. of estrogenic substance was found.) It was evident that after receiving injections of estrone, rats excrete considerably less estrogenic substance in the urine and feces than we found in our experiments with human beings.

Now the possibility presented itself that the injected estrone might be excreted in the urine as its hydrate, since it is known that the estrogenic activity of estrone hydrate (estriol) is only about one one-hun-

*We wish to thank the N. V. Organon Company for their kindness in supplying us with these substances.

dredth of the activity of estrone. On the other hand, the potency of the hydrate in regard to its ability to cause opening of the vagina of infantile rats is much greater than that of estrone. In order to determine whether estriol is present, two units of the estrogenic hormone from the urine, in solution in oil, were injected twice daily for two and one-half days, into a number of immature rats of the same age and weight. Control animals received two injections of two units each of crystalline estrone dissolved in oil. Since opening of the vagina occurred at the same time in most of the animals, the absence of significant amounts of estrone hydrate was proved.

B. CONTENT OF ESTROGENIC HORMONE IN THE WHOLE ORGANISM AFTER INJECTION OF ESTRONE

Since the urine and feces yielded only very small amounts of the injected estrone, the question arose as to whether estrogenic substance could still be detected in the body after the injections, and, if so, where. To solve this problem it was felt that a quantitative hormone extraction of the animal's entire body was of decided importance. Inasmuch as before this, we had performed only a few similar experiments we, therefore, at the beginning of this investigation, used three different methods of extraction: (1) Extraction with benzene after immediately adding acid. (2) Alcohol extraction: (a) without subsequent acid hydrolysis, and (b) with subsequent extraction in acid medium. (3) Benzene extraction after drying the minced animal with sodium sulphate. In all three extraction methods the whole animal was first ground in a mincing mill to a mash. The urine and feces collected from the beginning of the experiment were added to this. The estrogenic activity of the extracts obtained by these three different methods was all assayed in the usual manner in castrated mice.

Method 1.—The mash was mixed with water to a total weight of 500 gm. After adding 75 c.c. of 25 per cent aqueous hydrochloric acid and 200 c.c. of benzene, the mixture was refluxed in a round-bottomed flask on a water-bath; the benzene being renewed twice during the extraction. The combined benzene extracts were first washed with water, until the wash water had a neutral reaction, and then evaporated in vacuo. The residue was dissolved in oil and assayed in ovariectomized mice.

Method 2.—The mash was mechanically shaken for twenty-four hours in 4 volumes of 96 per cent alcohol and centrifuged. The supernatant alcohol was passed off and the remainder was shaken twice more with 500 c.c. of 70 per cent alcohol. The combined alcohol extracts were evaporated to dryness; the residue treated with a small amount of alcohol, and centrifuged. One part of this extract was used for testing (Method 2a); the remaining part was suspended in water. To each cubic centimeter of liquid, 150 c.c. of 25 per cent hydrochloric acid were added and extraction was performed for 3 periods of four hours each with one-third volume of benzene (the benzene being renewed twice). The combined benzene extracts were washed and evaporated.

Method 3.—The mash was mixed with an equal weight of anhydrous sodium sulphate and allowed to stand overnight. The next day the hard mass was powdered and boiled using reflux with benzene three times during four hours, the benzene being renewed each time. The combined benzene extracts were evaporated to dryness.

ANIMAL EXPERIMENTS

First the content of estrogenic material in the normal rat was determined. Extractions were carried out with three female rats, weighing between 170 and 210 gm., according to Method 3. The neutral extraction was followed by an acid one; after neutral extraction, 500 c.c. of 4 per cent hydrochloric acid were added to the residue and the mixture was boiled with benzene. None of the extracts, whether neutral or acid, showed any trace of estrogenic activity working on the assumption that 50 M.U. might be present in the whole animal. The amounts collected from the whole animal at different intervals after injections of estrone and estradiol are summarized in Table I.

a. *Free Estrone and Estradiol.*—Forty-eight hours after the injection of estrone and estradiol, the hormone content of the body, with one exception, was less than 10 per cent of the amount injected. Six hours after the hormone injections, an average of only 20 per cent of the amount of hormone injected could be recovered. However, one and two hours after the injections, as much as 70 to 80 per cent was still present. With the three methods already described, the same values were obtained. This proves that the hormones administered were not converted into a combined form, i.e., in a form in which they occur in human urine. The experiments with 1,000 and 10,000 M.U. gave exactly the same results.

b. *Investigations With Benzoate.*—With the benzoates the result was different. Forty-eight hours afterward, between 20 to 60 per cent was still recovered; approximately the same values were obtained twenty-four hours afterward (40 to 70 per cent). One to two hours after the injections, practically the total amount of hormone injected could be detected.

These results agree with the well-known protracted activity of the benzoate esters. In our experiments with hormone benzoates, we obtained the same quantities of hormone by all methods with and without hydrolysis. Since we know that the free hormone in equal quantity exerts an activity four times stronger than the corresponding benzoate, the question may arise whether a liberation of free estrone could have occurred during extraction, or in the body of the animal after administration of estrone benzoate. This, furthermore, would have accounted for a high estrogenic activity of the extract in spite of the loss of 75 per cent of the injected estrone benzoate.

A closer investigation showed that under the conditions chosen, the benzoic esters are not saponified in acid medium. In the first place, both esters are more easily saponified in alkaline than in acid medium; moreover, as in our Methods 1 and 2b, heating had only begun after the addition of benzene, the ester had passed into it already and so escaped hydrolysis. The following experiment proves this to be the case.

There were 5,000 M.U. of estrone benzoate dissolved in 10 c.c. of oil. To 4 c.c. of this oily solution (=2,000 M.U.) were added 50 c.c. of 96 per cent alcohol and 2 gm. of potassium hydroxide dissolved in a little water. After refluxing for four hours, the mixture was worked up in the usual way and the quantity of estrogenic material estimated biologically. As this amounted to 8,000 M.U. a complete saponification had taken place in this alkaline solution. Another 4 c.c. of the oil was refluxed with 500 c.c.

TABLE I. RECOVERED HORMONE QUANTITIES IN THE TOTAL ANIMAL IN DIFFERENT INTERVALS AFTER INJECTION*

NO.	ESTROGENIC HORMONE	QUANTITIES INJECTED SUBST. M.U.	QUANTITIES INJECTED SUBST. MG.	METHOD OF EXTRACTION	KILLED AFTER	HORMONE M.U.	RECOVERED PERCENTAGE
10	Estrone	1,000	0.1	I	48 Hr.	100	10
12	Estrone	1,000	0.1	III	48 Hr.	100	10
19	Estrone	10,000	1.0	IIa	48 Hr.	150	1.5
30	Estrone	10,000	1.0	III	48 Hr.	100	10
31	Estrone	10,000	1.0	III	48 Hr.	700	7
32	Estrone	10,000	1.0	III	48 Hr.	300	3
20	Estrone	1,000	0.1	IIa	6 Hr.	300	30
21	Estrone	1,000	0.1	IIa	6 Hr.	200-300	20-30
79	Estrone	10,000	1.0	I	6 Hr.	1,600	16
80	Estrone	10,000	1.0	I	6 Hr.	2,000	20
81	Estrone	10,000	1.0	I	6 Hr.	1,500	15
1	Estrone	10,000	1.0	I	1-2 Hr.	7,000	70
2	Estrone	10,000	1.0	IIa	1-2 Hr.	7,000	70
				IIb	1-2 Hr.	7,000	70
3	Estrone	10,000	1.0	III	1-2 Hr.	1,000	100
3	Estrone	1,000	0.1	I	1-2 Hr.	750	75
4	Estrone	1,000	0.1	III	1-2 Hr.	800	80
18	Estradiol	1,000	0.05	IIa	6 Hr.	200	20
19	Estradiol	1,000	0.05	IIa	6 Hr.	100	10
4	Estradiol	1,000	0.05	I	1-2 Hr.	700	70
5	Estradiol	1,000	0.05	IIa	1-2 Hr.	600	60
	Estradiol	1,000	0.05	IIb	1-2 Hr.	800	80
6	Estradiol	1,000	0.05	III	1-2 Hr.	750	75
26	Estradiol	1,000	0.05	IIa	1-2 Hr.	700	70
40	Estrone benz.	1,000	0.4	III	48 Hr.	600	60
51	Estrone benz.	2,000	0.8	III	48 Hr.	400	20
52	Estrone benz.		0.8	III	48 Hr.	500	25
39	Estrone benz.	1,000	0.4	IIa	24 Hr.	700	70
42	Estrone benz.	1,000	0.4	IIa	24 Hr.	500-600	50-60
15	Estrone benz.	1,000	0.4	I	1-2 Hr.	800	80
16	Estrone benz.	1,000	0.4	IIa	1-2 Hr.	1,000	100
				IIb	1-2 Hr.	1,000	100
17	Estrone benz.	1,000	0.4	III	1-2 Hr.	750	75
41	Estradiol-monobenzoate	1,000	0.125	III	48 Hr.	200	20
38	Estradiol-monobenzoate	1,000	0.125	IIa	24 Hr.	600	60
43	Estradiol-monobenzoate	1,000	0.125	IIa	24 Hr.	500	40
7	Estradiol-monobenzoate	1,000	0.125	I	1-2 Hr.	750	75
8	Estradiol-monobenzoate	1,000	0.125	IIa	1-2 Hr.	900	90
9	Estradiol-monobenzoate	1,000	0.125	III	1-2 Hr.	800	80

*Each dosage was tested in 3 mice (12-24 mice were used) for the complete assaying of each preparation.

of a 4 per cent aqueous solution of hydrochloric acid and benzene for three periods of four hours, the benzene being renewed twice. The combined benzene extracts were washed, evaporated, and the dry residue assayed. This proved to contain 2,000 M.U. of estrogenic hormone only. Under the above-mentioned conditions in this acid medium, no saponification had taken place at all. Repeated experiments yielded the same result.

The question now arises as to where and in what form the hormone which was recovered from the animal's body is found. This question is of great importance, particularly in regard to the benzoate. There are two possibilities: (1) the hormone is still in the original place of injection, i.e., it has not been absorbed, and (2) the hormone was absorbed immediately but is circulating in the body or temporarily has been stored somewhere in the body. In an attempt to find an answer to this question, the following experiment was carried out. There were 10,000 M.U. of estrone injected into a rat, and one hour later the animal was killed and divided into two parts. The part containing the site of injection weighed 48 gm.; the other part, 102 gm. Both parts were extracted according to Method 2a. In the former part (that with the site of injection), 8,000 M.U. of estrone were found; in the latter, 1,500 M.U. From these facts we may conclude that after one hour, only a small part of the estrone has been absorbed. Another rat received 10,000 M.U. of estrone and was killed one hour after the injection. But in this case the oil at the site of injection was removed with a piece of cotton wool and the area washed with ether. The piece of skin at the site of injection was removed and extracted together with the piece of cotton wool. The yield from this was 5,000 M.U. Further experiments were carried out in the following manner: the animals were killed at different intervals after the injection of estrone and estrone benzoate. The oil under the skin at the site of injection, together with a piece of the surrounding tissue, was extracted apart from the remainder, according to Method 2a. The results are listed in Table II.

It is evident that six hours after the injection of free estrone, an average of 10 to 25 per cent of the estrone was recovered from the site of injection; whereas, following the same lapse of time after the injection of the ester, 80 to 90 per cent was recovered. Twenty-four hours after the injection 50 per cent of the ester had not yet been absorbed. In one case, 15 per cent was still found under the skin forty-eight hours after the injection.

In the body itself, with the exception of one rat (No. 65, in which 6,000 M.U. were found), very small amounts of hormone or ester were discovered. We may assume that with this rat the site of injection had not been indicated correctly so that the greater part of the ester which had not been absorbed was extracted with the rest of the body. From

the experiments with estrone it appears clearly that, after six hours, no more than 25 per cent of the amounts injected was present at the original site of injection, while the remaining 75 per cent could not be detected in the animal organism.

TABLE II

NO.	ESTROGENIC HORMONE	QUANTITY OF E. H.		ANIMALS SACRIFICED AFTER: HR.	HORMONE FOUND		TOTAL AMOUNT OF HORMONE	
		UNITS	MG.		AT SITE OF INJECTION	IN THE RAT	UNITS	%
55	Estrone	10,000	1.0	6	2,500 (25%)	500	3,000	30
56	Estrone	10,000	1.0	6	2,500 (25%)	1,000	3,500	35
65	Estrone	40,000	4.0	6	500 (1½%)	6,000	6,000	+15
75	Estrone	20,000	2.0	6	2,000 (10%)	----	----	--
57	Estrone	1,000	0.1	24	100 (10%)	100	100	10
76	Estrone benzoate	5,000	2.0	6	4,500 (90%)	----	----	--
59	Estrone benzoate	1,000	0.4	6	800 (80%)	<200	±800	±80
60	Estrone benzoate	1,000	0.4	6	800 (80%)	<200	±800	±80
45	Estrone benzoate	1,000	0.4	24	500 (50%)	<100	500	50
53	Estrone benzoate	1,000	0.4	24	500 (50%)	<100	500	50
54	Estrone benzoate	1,000	0.4	24	800 (80%)	<100	800	80
49	Estrone benzoate	1,000	0.4	48	150 (15%)	100	----	--
56	Estradiol benzoate	1,000	0.125	48	100 (15%)	<100	----	--

The problem then arises, "What happens to the absorbed estrone?" In the first place the possibility of conversion of the estrone after absorption into an esterified form (which has one-fourth of the physiologic potency of the estrone) had to be excluded. If we assume an esterification two possibilities arise. There could have been formed a lipoid soluble ester or a lipoid insoluble one similar to the form excreted in human urine. The latter possibility could be excluded since with our different methods of extraction (neutral as well as acid) the same values had been obtained, which results could be confirmed in control experiments. The problem was elucidated in the following way (Table III). As usual the rat was extracted in neutral medium, the free hormone as well as its lipoid soluble ester, if formed, passing into the benzene phase. Now the mash was strongly acidified and extracted under reflux with benzene for four hours. In this manner any lipoid insoluble ester present is hydrolyzed. The hormone, thus liberated, would be readily recovered by the subsequent benzene extraction. In order to prove a possible formation of lipoid soluble ester, a part of the residue obtained

TABLE III. THE ANIMALS WERE KILLED SIX HOURS AFTER INJECTION

NO.	INJECTION ESTRONE	FOUND UNDER SKIN		AFTER NEUTRAL EXTRACTION OF RAT		AFTER ACID EXTRACTION OF RAT
		BEFORE HYDROLYSIS	AFTER HYDROLYSIS	BEFORE HYDROLYSIS	AFTER HYDROLYSIS	
7	10,000 M.U. 1mg.	----	----	1,000	1,000 U	
9	10,000 M.U. 1mg.	1,000	1,000	500	500	<50 M.U.
10	10,000 M.U. 1mg.	2,000	2,000	500	500	<50 M.U.
11	10,000 M.U. 1mg.	1,000	1,000	200	200	100 M.U.

from the neutral extract was refluxed for four hours with alcoholic potassium hydroxide. The bulk of the alcohol was evaporated and the residue dissolved in water, acidified and extracted with benzene. The free estrone should be found in the latter. No increase of the estrogenic activity after hydrolysis could be found in the skin or in the mash. The same proved true after acid hydrolysis of the mash which had been previously extracted in neutral medium.

One more possibility still remained to be investigated, viz.: whether the hormone injected circulates through the body in the form of its hydrate. In order to detect this compound, the potency of the extract in regard to its capacity to cause opening of the vagina was determined in infantile rats. The method of doing this has already been described in connection with the urine studies. Judging from the amount of estrogenic units necessary to cause opening of the vagina it appears that the estrogenic substance was not estriol. These results confirm the observations of Zondek.

As regards the possible fate of injected benzoates of estrone and estradiol, the following possibilities must be considered: (1) The benzoate is hydrolyzed by enzyme action liberating estrone. (2) The benzoate is absorbed and is circulating as such through the body. (3) The ester is hydrolyzed elsewhere in the body, the freed hormone then exerting its action. (4) The freed hormone is converted again into an ester in the body but into a lipoid insoluble one.

All these possibilities have been investigated.

1. Two rats (Nos. 70 and 71) receiving 10,000 mouse units (4 mg.) and two rats (Nos. 87 and 88) receiving 5,000 mouse units (2 mg.) of estrone benzoate were killed six hours after the injection. The oil together with the surrounding tissue was removed in the usual manner. After neutral extraction the benzene extract was assayed for its estrogenic activity. In Rats 70 and 71, 7,500 and 7,000, while in Rats 87 and 88, 3,750 and 3,000 units, respectively, could be found at the site of injection, unabsorbed. Parts of these four neutral extracts, equivalent to 1,000 estrogenic units, were evaporated, the residues dissolved in 50 c.c. of 50 per cent alcohol and boiled under reflux for four hours (after adding potassium hydroxide). The bulk of the alcohol was evaporated, the residues were diluted with water and acidified with hydrochloric acid to Congo red. The acid solution was shaken four times with benzene. The estrogenic activity of the benzene extracts was then assayed. After hydrolysis, 3,000 and 3,500 mouse units, respectively, were found in the four extracts. (As is known, the international unit of estrone is 0.1 gamma. In this laboratory the equivalent amount of estrone benzoate is found to be 0.4 gamma.) Accordingly, after hydrolysis of 1,000 estrogenic units (0.4 mg.) of estrone benzoate, we obtain theoretically 4,000 mouse units of estrone. In the above experiments we found an increase of the action from 1,000 to 3,000 and 3,500 mouse units. In other experiments we found a fourfold increase in potency. From these results we may conclude with certainty that the part that had not been absorbed still consists of unaltered benzoate.

2. After removing the parts containing the nonabsorbed hormone, the bodies of the rats (Nos. 70, 71, 87, 88) were minced and after drying with anhydrous sodium

sulphate were extracted with benzene, according to Method 3. In the extracts of Rats 70 and 71, 1,500 and 2,500, and in those of Rats 87 and 88, 600 and 1,200 mouse units, respectively, were found. Parts of these rat extracts, equivalent to 1,000 estrogenic units, were boiled with reflux with alcoholic potassium hydroxide for two hours. From the alkaline solution, benzene extracts were obtained in the manner already described. After this alkaline hydrolysis, 800 to 1,000 mouse units could be recovered. This indicated that the substance circulating through the body after the injection of estrone benzoate is no longer this ester but probably, for the greater part, free estrone.

3 and 4. Finally it was shown that after the injection of estrone benzoate no increase in the amounts of lipoid insoluble estrogenic hormones are formed in the organism. Therefore, the minced specimens of lipoids after neutral extraction were boiled in strong acid solution for four hours. (It is known that the bound hormone occurring in urine is hydrolyzed in acid medium.) The acid mixtures were extracted with benzene for three periods of four hours each, the benzene being renewed each time. The combined benzene extracts were assayed. After this hydrolysis in the mixed specimens of Rats 70, 71, and 87, less than 100 mouse units of estrogenic substance could be found. The acid benzol extract of Rat 88, however, contained 200 mouse units. After neutralization each mass was boiled with alcoholic potassium hydroxide for four hours, all the proteins being thus hydrolyzed. The extracts were worked up as usual and in all cases proved to contain less than 50 units. The results are summarized in Table IV.

TABLE IV

NO.	QUANTITY OF ESTRONE-MONOBENZOATE		KILLED AFTER HOURS	UNITS FOUND UNDER THE SKIN		IN THE RAT AFTER NEUTRAL		ACID EXTRACT
	UNITS	MG. SUBSTANCE		BEFORE HYDROLYSIS	AFTER HYDROLYSIS	BEFORE HYDROLYSIS	AFTER HYDROLYSIS	
70	10,000	4	6	7,500	3 × 7,500	1,500	1,500	100
71	10,000	4	6	7,000	3.5 × 7,000	2,500	2,500	100
87	5,000	2	6	3,750	3.2 × 3,750	600	± 600	100
88	5,000	2	6	3,000	4 × 3,000	1,200	±1,200	200
82	2,500	1	48	100	3.5 × 100	65	± 65	50
83	2,500	1	48	150	4 × 150	100	±100	50
84	2,500	1	48	200	3.5 × 200	65	65	50

In the body neither lipoid soluble nor lipoid insoluble esters were found. In the former case, an enhanced estrogenic reaction was to be found after saponification of the neutral extract from the mash; in the latter case free hormone must have been detected in the acid extract which was obtained after the neutral extraction of the mass. A protracted action of the benzoate ester is certainly not caused by a slow cleavage of the acid component in the body.

The longer period of estrogenic effect noted after the injection of the benzoate ester into ovariectomized mice is due to its delayed resorption.

Where in the body the estrogenic substances are inactivated still remains an open question. According to Zondek, mashed liver could inactivate estrone in vitro. Our preliminary experiments did not confirm this assumption; further investigations are to be continued.

SUMMARY

1. After administering large amounts (2.5 mg.) of estrone to rats only very small quantities were recovered in the urine and feces which were collected for eight days after the injection. For this reason the hormone content of the whole animal was investigated.

2. During this investigation three different extraction methods were used and gave the same results.

3. After injecting estrone and estradiol a small part only was recovered from the animal body six hours afterward.

4. Six hours after administering estrone benzoate the bulk of it could be recovered unaltered, while often 50 per cent of the estrogenic units had not yet been absorbed as long as twenty-four to forty-eight hours after the injection.

5. After the injection of the benzoate ester this substance does not circulate through the body as such but as free hormone.

6. The protracted action of the benzoate is due to its delayed absorption and not to the slow hydrolysis of the ester in the body.

7. After administering estrone or estradiol, lipoid insoluble estrogenic hormones similar to those occurring in the urine, could not be found.

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THE STANDARDIZATION OF ANTERIOR PITUITARY HORMONES

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SINCE Evans[1] first showed that anterior pituitary extracts could influence growth, many reports of studies upon the physiologic effects of the implantation of anterior pituitary substance or of the injection of various extracts of the fresh or preserved glands have appeared. In fact, in recent years the number of papers dealing in one way or another with this important subject has been growing almost by geometric progression. A critical analysis of these various contributions shows beyond any doubt that many clear-cut and distinct physiologic effects can be produced by anterior pituitary extracts. The question has arisen repeatedly in the mind of the individual worker as to whether the apparent specific effect of a certain extract is due to a specific anterior lobe hormone or active principle or to an active principle which has in addition other physiologic activities. Since the actual properties of anterior lobe extracts are so many and so varied, it becomes on a priori reasoning a matter of grave doubt that the number of active principles can equal the number of known specific effects. The writer has long since come to the conclusion that irrespective of the number of physiologic effects which may be demonstrated for various anterior lobe preparations, and irrespective of the apparent purity in a physiologic sense of this or that extract, the number of active principles in the normal gland in the living subject must of necessity be few (three or four). I do not believe that anyone has as yet obtained an anterior lobe extract without some modification of the active principle or principles present in the living gland having taken place. Although I have no very strong evidence for it, I like to visualize the naturally occurring anterior lobe hormone or hormones as rather large protein molecules to each of certain prosthetic groups of which some specific physiologic effect may be related. Such a view allows at least of one cherishing the hope that some day simpler compounds having specific physiologic effects may be obtained in crystalline form. These would not be true hormones but breakdown products resulting from controlled hydrolytic or other processes yet to be discovered.

Meanwhile, even though the number of effects of anterior pituitary extracts is still increasing, it becomes most essential that some system of biologic standardization of extracts should be agreed upon, particularly

so that experimental clinical studies can be satisfactorily made and the results of such adequately evaluated. At the present time, there is not enough information to allow of the setting up of absolutely rigid standards, but an attempt can be made in this direction, and in the course of time, as more exact knowledge becomes available, methods of testing which are universally acceptable may be agreed upon.

DISCUSSION OF ASSAY METHODS

GROWTH

Exact assay of the growth principle is a matter of great difficulty for various reasons. Probably the best test object is the recently hypophysectomized rat weighing about 100 gm. The minimum amount of extract given twice daily by subcutaneous injection which will result in an average gain in weight of 1 gm. per day over a period of ten to fifteen days may be taken as a unit. Normal rats whose growth curves have tended to plateau may be used also according either to the method of Evans and Simpson[2] or Lee and Schaffer.[3]

Even though by the use of a sufficiently large number of animals an apparently satisfactory assay has been made on any one particular extract, there still remains the possibility that the growth principle present may have been enhanced or inhibited by the presence of other substances in the extract under test. Difficulties such as these are bound to be encountered not only in the assay of the growth principle but also of other principles until such time as the individual active substances are obtained in relatively pure form. In a growth extract made by the "Q" process there is probably a minimum of other active principles.[4] This extract is impractical, however, since the yield of active substance may be as low as 5 per cent. On the other hand, a simple alkaline extract containing small amounts of practically everything in the original gland tissue may cause a positive growth response in the hypophysectomized rat when the equivalent of as little as $\frac{1}{2000}$ gm. of pituitary is given per day. Again certain extracts rich in thyreotropic hormone may appear to have little or no growth effect when tested on hypophysectomized rats but a "Q" fraction may be obtained from such a mixture by the use of $Ca_3(PO_4)_2$ which can be shown to have considerable growth-promoting power.

THE THYREOTROPIC PRINCIPLE

The thyroid-stimulating principle of anterior lobe extracts can be assayed according to one or other of various criteria with a fair degree of accuracy, but here again, as in the case of the growth hormone, one cannot be sure in dealing with any one extract containing this substance whether the assay value obtained represents an increase or a

decrease over the exact value due to the synergistic or inhibitory action of associated substances. There are several fairly exact methods for measuring the potency of an unknown extract to influence the thyroid gland. Unfortunately, however, the results obtained by one method, even though they may be readily duplicable, may not parallel the results obtained by a different method. Observations of this kind, while at the moment very confusing, point very strongly, as Heyl and Laqueur,[5] and Collip[6] have suggested, to the possibility that the anterior pituitary may influence the thyroid gland in more than one way. The methods of assay which have been suggested for the thyroid stimulator may be classified as follows:

1. Those depending upon the increase in the thyroid weight following treatment according to a definite schedule.
2. Those depending upon change in histologic structure.
3. Those depending upon changes produced in metabolism.
4. A method depending upon changes in the iodine content of the thyroid gland.

THE INDIVIDUAL METHODS OF ASSAY

1. Rowlands and Parkes[7] make use of the percentage increase in the weight of the thyroids of guinea pigs weighing 200 gm. The extract to be assayed is injected daily for six days. Controls consist of untreated animals and animals treated with a standard extract. The unit is taken as the amount which causes a 50 per cent increase in the thyroid weight during the time of the test. It is essential for accurate work that the pigs should be raised under well-controlled conditions of housing, feeding, temperature, etc.

Riddle[8] has found that the young dove or pigeon may be used to advantage for thyreotropic hormone assay, but as yet there has been no attempt to correlate the bird and the guinea pig unit.

2. The production of hyperplasia as demonstrated histologically or cytologic changes associated with hyperfunction has been a favorite method with various workers (Junkmann and Schoeller,[9] L. Loeb and Friedman,[10] Krogh and Okkels,[11] etc.).

The histologic method recently described by Heyl and Laqueur[5] is in our opinion the most accurate as well as serviceable of this type: 200 gm. guinea pigs are injected intraperitoneally on each of two days and killed at forty-eight hours. The detection by histologic study of the first delicate changes which occur in the acinar cells is made the basis for a positive reaction.

The restoration to normal of the atrophic thyroid gland of the hypophysectomized rat can be used as an alternative method of assay in this group, dependent upon structural change demonstrable by microscopic examination. When this method is made use of, the animals should be of a certain age group and used at a definite time after hypophysectomy.*

3. The increase in metabolism of the hypophysectomized rat operated upon at a definite age and otherwise untreated gives a very satisfactory criterion for assay of the factor in anterior lobe extracts responsible for the elevation in metabolic rate.

*Another method which has much to commend it has been reported by Starr and Rawson at the Atlantic City meeting of the American Society for Clinical Investigation, May 3, 1937. It is a micrometric analysis of the response of the guinea pig thyroid.

We have suggested as a unit, using this method, the minimum amount which when injected subcutaneously twice daily for three days causes an increase in metabolism of 20 to 25 per cent.

Because this method presents many difficulties, we have been attempting to correlate with it a similar procedure in which normal guinea pigs weighing 200 gm. are used. We are endeavoring as well to correlate histologic change and thyroid weight increase both in hypophysectomized rats and in normal pigs with the metabolic response of each. It is too early to draw final conclusions from this work, but it is our impression that ultimately assays will have to be made both for the metabolic effect and for the thyroid enlarging effect since we have noted on several occasions marked differences in regard to these two effects in the same extract. These observations suggest that the enlargement of the gland and the release of thyroid hormone are two separate and distinct functions. If this proves to be true, then the problem of clinical goiter in relation to the anterior pituitary will tend to become less obscure.

4. McCullogh and Stimmel[12] have suggested as an alternative method of assay for the thyreotropic principle the decrease in the iodine content of the gland of the treated animal. This method, which is only available to experts in iodine determination, should prove of great value in the solution of the problem suggested in the previous paragraph.

THE GONADOTROPIC PRINCIPLE

There are numerous methods of demonstrating the maturity or gonadotropic activity of anterior pituitary substance or extracts. Thus either males or females of widely divergent species may be used. From my own experience I prefer to use the immature female rat. The immature male, the prepubertal or postpubertal hypophysectomized male or female rat, may also be used. The immature male dove or pigeon has been shown by Riddle[8, 13] to be an excellent test object for follicle-stimulating hormone. Many others use the ovulation test in the estrous doe. Laboratory studies generally with the gonadotropic substance are complicated by the fact that there probably are two factors involved at least in the case of the female, one the so-called follicle-stimulating hormone (F. S. H.) and the other the luteinizer or L. H. A full discussion of this topic is not indicated here; suffice it to say that in view of the apparent synergistic action between these, it is practically impossible in the light of the work of Fevold and Hisaw[14] to obtain an absolute value for the F. S. H. content unless the extract being tested is free of L. H.

The usual effect of treatment by subcutaneous injection of immature female rats with a good preparation of gonadotropic substance is a rapid increase in ovarian size, which reaches a maximum in from seventy-two to ninety-six hours. Since the upper limit of ovarian weight does not as a rule exceed 60 mg., irrespective of the size of the dose administered, I have suggested as a laboratory unit of gonadotropic potency the minimum amount which, injected daily in three divided doses for three days, produces ovaries of 30 to 40 gm. in weight in

seventy-two hours.* The rats used for assay are twenty-one days old at the beginning of the test. In order to bring the clinical unit for this substance more in line with the placental hormone of pregnancy blood and urine (A. P. L.), I suggest that the clinical unit be one-tenth of that defined above. Many variations of the immature rat test have been used and reported from different laboratories. Thus injections may be made once, twice, or three times daily; vaginal smears can be taken and if one wishes can be made the criterion of activity. One point of extreme importance, irrespective of which modification of the rat test is being used, is that the injections be made subcutaneously. Intraperitoneal injections are absolutely useless if an accurate assay is to be made.

ADRENOTROPIC SUBSTANCE

The adrenotropic substance in our experience cannot be satisfactorily assayed on other than an hypophysectomized animal, preferably the rat. The restoration toward normal of the atrophic cortex of the hypophysectomized rat is at present the method of choice for the determination of adrenotropic potency. Large doses of adrenotropic extracts may produce enlargement of the suprarenal cortex of the normal rat but since so many nonspecific agents have been shown to produce a similar result (probably through the release of adrenotropic substance from the intact pituitary) I consider it absolutely unsafe at this time to use the normal animal for this purpose. Unfortunately, no test based on a physiologic response has been discovered as yet. The technic of carrying out the test as originally described is as follows:

The left adrenal is removed from hypophysectomized rats ten days to two weeks following the operation. This gland is weighed and sectioned for microscopic examination. The animal is injected twice daily for six days with the extract to be tested. The animal is killed and the right adrenal is weighed and sectioned, as is also the thyroid gland. Control experiments have shown that there is no compensatory hypertrophy of the remaining gland after unilateral adrenalectomy in the hypophysectomized animal. Increase in weight of the remaining adrenal over that of the one taken at biopsy, together with microscopic evidence of cortical repair, is taken as positive evidence of adrenotropic activity in the extract. The minimum amount of extract given daily which caused a 50 per cent increase in the weight of the gland was taken as the unit.

A modification of this test consists in using a larger number of hypophysectomized animals and eliminating the removal of one gland. Since it may be assumed that the weight of the glands in untreated

*A new method for the assay of follicle-stimulating substances has been reported recently (Annual meeting of the Federation of Biological Societies, Memphis, Tenn., April 22, 1937) by Levin and Tyndale. The effect of graded doses of a gonadotropic extract upon uterine weight of the mouse is used as the basis for assay.

animals will be from 9 to 12 mg., a dose of extract is found which will cause the glands to increase approximately 50 per cent in weight in a period of five days.

PROLACTIN

That substance present in certain anterior lobe extracts which appears to be purely a secretagogue to the fully developed mammary gland and which has been so extensively investigated by Riddle and his coworkers,[13] also by Gardner and Turner,[15] and others, is assayed by choice by the crop gland response of the immature dove or pigeon. This method was introduced by Riddle and has been standardized perhaps to a more satisfactory degree than is the case with most hormone assay methods. The unit, as it has been defined by Riddle, was based on the crop gland response in the two-month-old squab, comparison being made of the unknown with a standard preparation, 1 mg. of which was the threshold dose per 150 gm. of pigeon. Injections were made intraperitoneally daily for four days. It is of interest in connection with prolactin that the crop gland response has been shown by Riddle to vary tremendously depending upon the mode of administration of the extract. Subcutaneous administration is many times [as high as 20] as effective as intraperitoneal injection. My own experience with prolactin has been similar to that of Riddle. It is very important, therefore, in the assay of this anterior pituitary hormone, as in the case of the gonadotropic factor, that the site of injection be carefully controlled.

Lyons and Page[16] have shown that a local crop gland response can be induced by the intradermal injection of a very minute amount of an active extract over the site of the crop. This is the most sensitive test known for the hormone and is of great value for detecting the presence of prolactin. For the present, however, Riddle's method appears to me to be the most reliable for accurate biologic assay of extracts designed for human use.

There are other physiologic effects of anterior lobe extracts for each of which there are methods of testing. These methods allow of an approximate determination of the potency of the extract in regard to each of a variety of effects. Thus the so-called diabetogenic action of anterior lobe extracts can be examined by noting the effect upon blood and urine sugar and acetone bodies of standardized Houssay dogs which have been treated with the extract.[17] The ketogenic action of extracts can be studied independently of the blood sugar raising action by using the young normal fasted rat.[18-21]

Recently, O'Donovan, working in my laboratory, has shown that the injection of certain pituitary extracts may cause a sharp rise in the metabolic rate which, however, is very transitory, lasting for only a few hours. This effect has been obtained in thyroidectomized animals

and is apparently independent of the thyreotropic hormone. This type of metabolic response is best studied upon thyroidectomized animals.

Some other effects of anterior lobe extracts, such as those upon liver size and fat content, protein metabolism, skeletal form and architecture, and blood calcium and calcium metabolism are known and methods for study of all of these are available; but it is doubtful to my mind whether any of this group of reactions will be of practical value in relation to the standardization of anterior lobe extracts for clinical use.

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SARCOMA OF THE VULVA

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SCARCELY less thrilling than the discovery of some rare flower by the botanist or a new star by the astronomer is the detection of an unusual form of tumor by those interested in pathology. Furthermore, it is through the careful analysis of the unusual that we sometimes find an answer to our everyday problems. Hence, it was with some satisfaction that in the past year I chanced upon the two unusual examples of sarcoma of the vulva that comprise this report. These two cases included a liposarcoma of the labium majus and a lymphosarcoma of the clitoris.

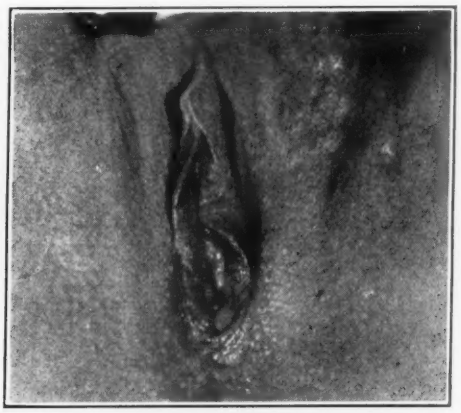
The best summary of our present knowledge of sarcoma of the vulva is to be found in the chapter contributed by E. Kehrer[3] to the *Veit-Stoeckel Handbuch*. Excluding, as seems to me quite proper, all cases of so-called melanosarcoma, he finds record of 77 cases of sarcoma developing from some portion of the external genitals. Anatomically data were available in 70 instances and these showed the following distribution: right labium majus, 23 cases (33 per cent); left labium majus, 20 cases (28.5 per cent); clitoris, 8 cases (11.4 per cent); labia minora, 3 cases (4.3 per cent); periurethral, 8 cases (11.4 per cent), and various combined lesions, 8 cases (11.4 per cent). In a small group comprising 14 cases collected by Nebesky[8] the sarcoma started in the urethra itself and extended to the vulva and vagina secondarily. B. P. Watson[12] also reports a case of this kind.

CASE REPORTS

1. *Liposarcoma of the Labium Majus*—

H. H., twenty-nine years of age, married, para i, came to the Barnard Free Skin and Cancer Hospital Aug. 11, 1936. She had no family history of malignant disease and had always been in good health, until the present illness. Four years previously at the age of twenty-five she had noticed a small hard lump in the left labium majus. This lump had suddenly grown larger in the past six months, and six weeks previous to coming to our hospital the tumor had been enucleated by another surgeon under the diagnosis of fibroma. Subsequent pathologic diagnosis of this specimen, concurred in by Leo Loeb, was liposarcoma. Two weeks after removal the tumor had recurred and on admission was 4 by 2½ cm. in size, adherent to the skin but freely movable over the underlying fat of the vulva and without ulceration (Fig. 1). In the left inguinal region a hard gland 1½ cm. in diameter could be felt. The patient was in excellent physical condition, had not lost in weight, and showed no evidence of metastasis at this time. In view of the well-known difference in method of metastasis, it was decided to abstain from the type of complete vulvectomy with extensive lymph gland resection (Basset operation) recommended for carcinoma of the vulva. Instead of this we did a one-sided vulvectomy removing merely the left-sided superficial inguinal and femoral lymph glands in continuity with the tumor. This operation was done Aug. 15, 1936 and the wound was completely healed five weeks later. Two months after this the patient began to have left lumbar backache and pains in the hips, spines, and shoulders. She was confined to bed at home. On Jan. 5, 1937 the temperature

was 100.6°; there was precordial pain and palpitation on exertion. At first it was thought these symptoms might be due to rheumatic fever, but it was soon evident that they were due to widespread metastases in the lungs, the bony structure, and probably also the valves and muscle of the heart. She was readmitted to the



A.

B.

Fig. 1.—*A*, Liposarcoma of the left labium majus. *B*, Operative specimen removed in continuity with the superficial inguinal and femoral lymphatics.

hospital January 20. The urine contained bacilli, considerable pus, albumin, and casts. An x-ray picture (Fig. 2) taken January 27 showed: "rib structures, lumbar spines and bones of the pelvis, including heads of both femurs, indicate unmistakable evidence of metastatic changes characterized by bone rarefaction. The lungs present

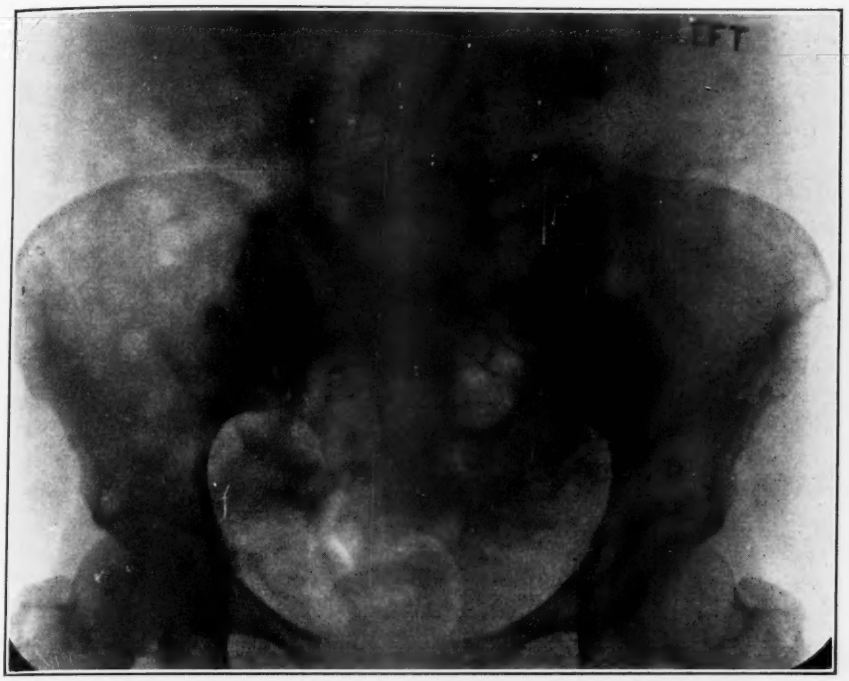


Fig. 2.—Roentgenogram of bone metastasis following liposarcoma of the vulva. Note especially the frayed-out lateral edge of the left ilium.

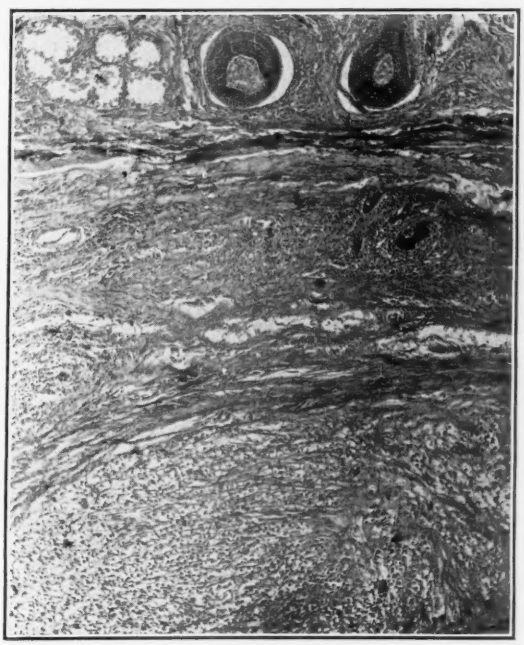


Fig. 3.—Low-power microphotograph of liposarcoma, showing definite fibrous capsule and separation from the skin (hair follicles visible).

lesions that are probably associated with metastasis.'' A blood transfusion of 500 c.c. given January 30 on account of progressive anemia availed but little. Pneumonia developed and death ensued Feb. 7, 1937. Autopsy was refused.

Pathologic report (Dr. Louis H. Jorstad): ''Specimen is one-half of the vulva. At midpoint is a tumor pushing up the skin and on section this is a mass measuring

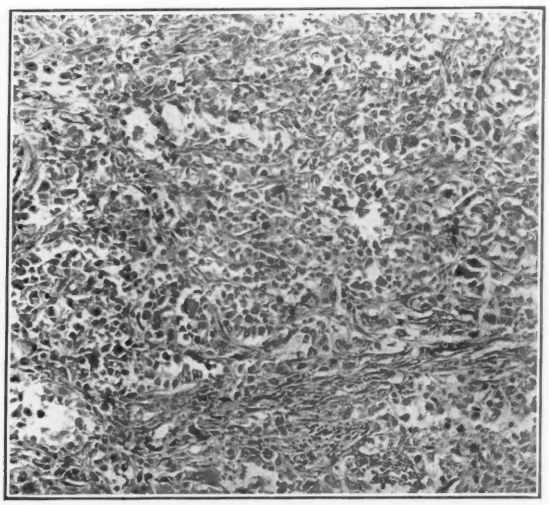


Fig. 4.—Section of liposarcoma, stained with hematoxylin and eosin. Magnified 90×.

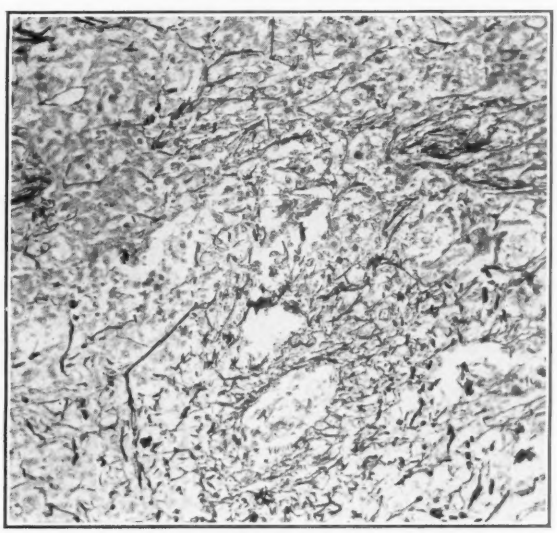


Fig. 5.—Section of liposarcoma, stained by the Foot-Bielschowsky silver impregnation method for reticulum fibers. Magnified 200×.

3½ by 2½ cm., situated in the vulvar fat, not attached to the skin (Fig. 3), for the most part opaque in color, with areas, however, of fatty color. It has somewhat of an alveolar arrangement. Along with the vulva is a mass of fatty tissue containing a number of lymph nodes. These are not particularly increased in size, are soft and quite normal in color.''

Microscopic diagnosis: Liposarcoma with hyperplasia of lymph nodes. Sections sent to Dr. James Ewing confirmed this diagnosis.

"Sections of the tumor stained with hematoxylin and eosin show a moderately rich cellular neoplasm separated into smaller and larger masses of fine and coarse bands of fibrous tissue (Fig. 4). The tumor cells are oval to spindle in shape, vary markedly in size, the nucleus is small, and the cytoplasmic structure contains fat globules of varying size and number. The cytoplasm is finely granular. These cells represent varying stages in the development of fat cells. The reticulum of the tumor demonstrated by the Foot modification of the Bielschowsky silver impregnation method is abundant in quantity and mainly in the form of a plexus surrounding each cell, the whole forming a network (Fig. 5). The intracellular fat was demonstrable in the frozen section, stained with Sudan III. The lymph nodes present a moderate hyperplasia, with no evidence of tumor cells."

DISCUSSION

Liposarcoma has been found in the uterus, the mammary gland, the kidney, bones and extremities. Kehrer[3] describes large and small round cell sarcoma, fibrosarcoma, myxosarcoma, and fibromyxosarcoma of the vulva. Only one case reported by Kleeberg[4] is described as a lipofibrosarcoma. According to Robert Frank[2] the histology of sarcoma of the vulva is varied, myxosarcoma, spindle-, and fibrosarcoma being most frequent. It seems not unlikely that with modern staining methods a few of these cases would have shown evidence of rising from the fatty tissue, especially in view of the predominence of tumors rising from the labia majora (61 per cent of the total). According to A. F. B. Shaw[10] the characteristic features of liposarcoma are: (1) they occur in adult life of either sex; (2) they are slow growing for many years with sudden increase; (3) encapsulation, readily removable at operation, does not suggest malignancy; (4) local recurrence usual, often repeated; (5) death follows metastasis; and (6) varied histologic structure. This case followed closely the characteristic clinical course of these tumors.

2. *Lymphosarcoma of the Clitoris*—

B. B., sixty-three years, married, 9 children, consulted me September 25, 1936. Menopause at forty-five years, gradual, without symptoms. Patient said that eight months previously she had noticed two small lumps the size of a lima bean above the urethra. These lumps were not tender or painful and gradually increased to the size of a small lemon. For two months the tumor had become irritated and bled a little. No pruritus. Some burning after urination. Weight loss of 8 pounds in the past year. A small lump was noted by the patient in the right groin one week before consulting me.

Examination showed a rather feeble old woman with marked evidence of myocardial degeneration (intermittent, rather rapid, pulse), blood pressure 168/70, moderate emphysema and arteriosclerosis. Locally there was seen a hard nodular mass involving the entire clitoris and a portion of the right labium minus (Fig. 6). The tumor was evidently subepithelial in origin but at one point over the clitoris had penetrated through the skin with formation of an ulcer 1 cm. in diameter. The labial extension also showed an area of ulceration as seen in Fig. 7. The urethra on the other hand was entirely free of direct involvement, as were also the left labia. Internal exploration revealed normal pelvic organs and vagina, without palpable iliac

gland involvement. In both groins there were numerous enlarged lymph glands and one hard femoral gland on the right side seemed definitely the seat of a metastasis. The clinical diagnosis of cancer of the clitoris was made. It was decided to do a vulvectomy and, if conditions permitted, to follow this two weeks later by a Basset removal of the tributary lymph glands.

On Sept. 28, 1936 at the Jewish Hospital the entire vulva was removed under local anesthesia. The patient made a satisfactory recovery from this operation. When the report came back that the tumor was a lymphosarcoma, it was thought wiser in view of this fact and the poor physical condition of the patient to be content with a simple superficial removal of the evidently enlarged glands on both sides. This was done Oct. 12, 1936. With the exception of the usual partial sloughing of

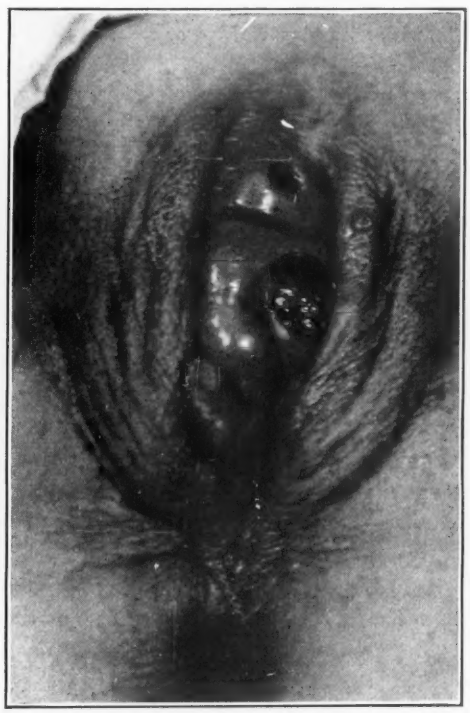


Fig. 6.—Lymphosarcoma of the clitoris, involving the prepuce and right labia with perforation ulcers.

the inguinal wounds the patient seemed on the way to recovery and was allowed to be out of bed for a short time after the twelfth day. On October 27, fifteen days after the second operation, while seated in the wheel chair, she suddenly collapsed, became cyanotic and died in thirty minutes, evidently as a result of embolism. Autopsy was refused.

Pathologic report (Dr. Sam Gray). *Microscopic:* Specimen consists of an excised vulva measuring 9 by 13 by 3 cm. The clitoris consists of a firm, rounded, red mass measuring 2 by 1½ cm. The frenulum and prepuce are well defined, the latter being quite edematous and containing two small, firm nodules, one of which is reddened and ulcerated. Just above the clitoris is a round, firm mass 3 cm. in diameter, presenting an ulcerated punched-out area in the center. Just above this nodule, deep in the subcutaneous tissues, is another firm area which, on cut surface, is irregular, white and firm. The right labium minus is indurated and discolored

and has an ulcerated area with a firm base, 1½ cm. in diameter. No other areas of induration are found in the specimen, the undersurface consisting of fat and subcutaneous tissue.

Microscopic: The section is that of the clitoris, the surface of which presents intact normal epithelium (Fig. 8). Just beneath the epithelium there is a dense aggregate of round cells, which are individual and show no syncytial arrangement. The majority of the cells are medium-sized lymphocytic structures with pale colored sparse cytoplasm. The nuclei are slightly irregular in outline, have a well-defined nuclear membrane and moderate amounts of chromatin loosely arranged in strands connecting irregularly shaped condensed masses. Nucleoli are frequently present and are irregular in outline. Occasional pyknosis of the nuclei is present. Mitotic figures are infrequent. Normal lymphocytes and plasma cells are rare. Typical

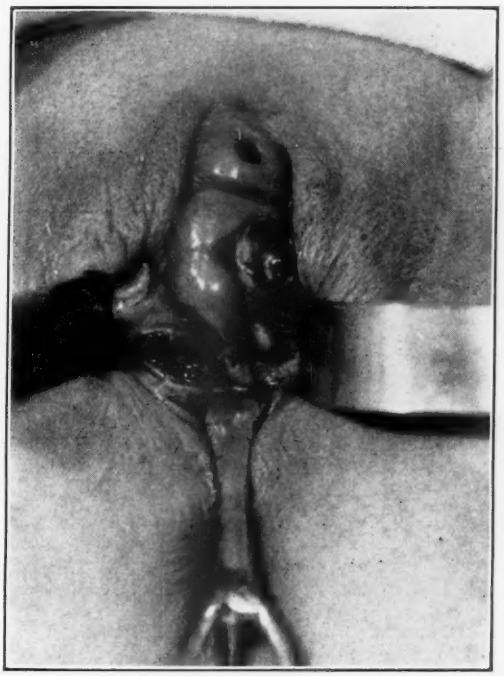


Fig. 7.—Same case as Fig. 6 with retractors in position to show that the tumor did not involve the urethra or vagina.

reticulum cells, with large vesicular nuclei containing fine strands of chromatin are occasionally encountered.

The morphology of the predominant cell type simulates the reticulum cell of the loose connective tissue more closely than the lymphocyte. Transitions between the two extremes can be found but the majority of the tumor cells show relatively slight differentiation from the more primitive reticulum (or mesenchymal) type of cell.

There is no evidence to suggest that this neoplasm is epithelial in origin. The epithelium is intact. The cells show no syncytial tendency. The slight amount of differentiation that is present is in the direction of a lymphocytic type of cell (Fig. 9). Diagnosis: Lymphosarcoma of clitoris.

Examination of the lymph glands removed at the secondary operation October twelfth showed merely normal hyperplasia with the exception of the large hard gland in the right femoral region which was filled with a metastasis showing the identical structure described in the primary tumor.

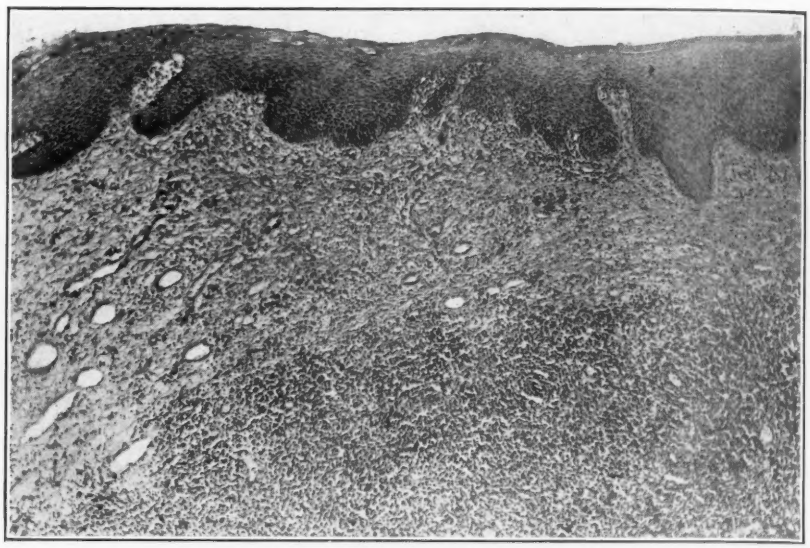


Fig. 8.—Section of lymphosarcoma of the clitoris, showing normal vulvar epithelium overlying the tumor. Magnified 110×.

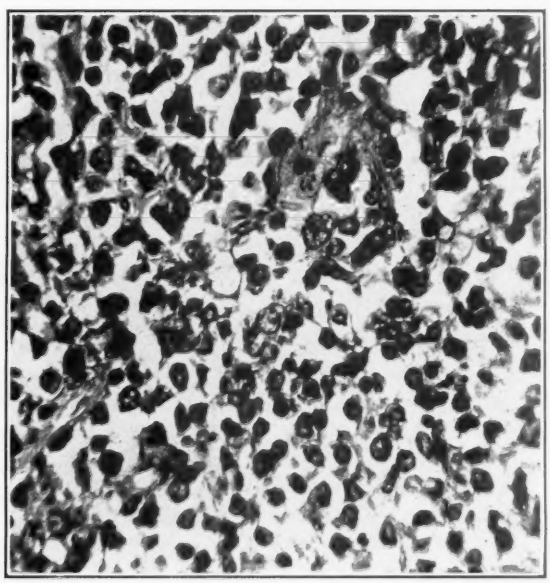


Fig. 9.—High power photomicrograph of lymphosarcoma of the clitoris, showing definite resemblance of the tumor cells to lymphocytes. Magnified 500×.

DISCUSSION

The designation of this tumor as a lymphosarcoma certainly seems justified in spite of the fact that it apparently represents the only tumor so classified to be found in medical literature. The changes that have recently taken place in the classification of malignant tumors may ac-

count for this, since doubtless many cases reported as round-celled sarcomas, if reviewed at the present time, would be classified as lymphosarcoma. It is a striking fact that tumors springing from within the substance of the glans clitoridis (not those, however, that develop from

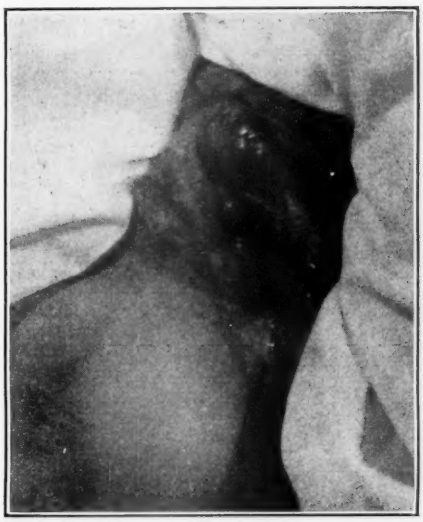


Fig. 10.—Anaplastic carcinoma of the glans clitoridis. Note the encapsulated subepithelial character of the tumor. Compare with Fig. 6.

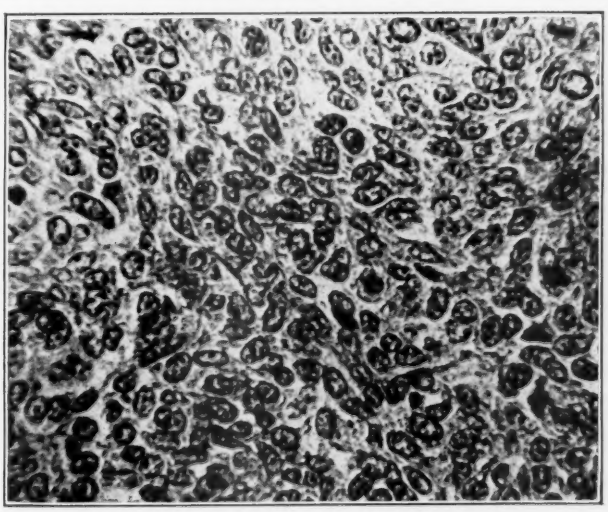


Fig. 11.—Section of anaplastic carcinoma of the clitoris, showing loose structure, many mitoses, but definite epithelial origin of cells. Magnified 500×.

the overlying preputial skin) have proved to be highly malignant. I[11] called attention to this fact some years ago in discussing carcinoma of the clitoris. Such tumors are often sarcoma-like, anaplastic in structure as seen in the section taken from a carcinoma of the clitoris (Figs. 10

and 11). It will be noted that the cells in this case have in areas a very loose structure with many mitotic figures. Nevertheless if this picture is compared with the microphotograph of the lymphosarcoma, the difference between the two cases will be apparent. It was only after a careful study that I became convinced that we were justified in grouping this case as a sarcoma rather than an anaplastic carcinoma.

CLINICAL ASPECTS OF SARCOMA OF THE VULVA

These two cases fit in closely with the clinical course of sarcoma of the vulva as previously described. Robert Frank[2] says: (p. 125) "They resemble fibromata until ulceration and infiltration takes place. . . . Early tendency to recurrence is the rule and multiple metastases may develop. The lymphatic glands are rarely affected, thus differing from carcinoma and melanoma." Frank Lynch[5] considers the prognosis grave. Death appears to result uniformly in cases in which the diagnosis of vulvar sarcoma is firmly established. In five cases complicated by pregnancy E. Kehrer[3] found that in none did the gestation seem to influence the growth of the tumor. In Maly's case the tumor was noted sixteen years before it suddenly grew larger and showed evidence of malignancy. In Blair Bell's[1] analysis of 18 cases of true sarcoma (not melanotic) of the vulva, the age distribution followed approximately that in cancer, the most frequent period being between thirty and fifty years. He found the myxomatous type of tumor the most common.

The diagnosis is usually made only after the disease is far advanced; hence, the treatment is usually very unsatisfactory. Surgery of the primary tumor is usually preferable to radiation and is ordinarily not attended by any difficulties. Only one case is on record where a five-year freedom from recurrence was reported (Maas-Olshausen).[6] Even after five years there may be a recurrence (Rhomberg).[9] The value of radium or deep x-ray therapy seems very questionable. Only a few cases have been treated by this method. All in all, sarcoma of the vulva presents at the present time a rather hopeless picture.

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THE TECHNIC OF TIMING HUMAN OVULATION BY PALPABLE CHANGES IN OVARY, TUBE, AND UTERUS*

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REASONING from a few cases studied intensively[1] and having happened upon occasional midinterval changes, I offer some tentative statements on the diagnosis of the time of ovulation and the organization of clinical research on this matter. Questions of methodology seem to be important because skepticism or failure of others in checking the findings may involve certain features that bear on confirmation. The first is one which only an Ancient can fully evaluate, namely, a degree of limitation in capacity for full visualization through the fingertips. This may be due to the lack of that training which we had in the early days of gynecology, that prelaparotomy elaboration of bimanual diagnosis forced upon us through absence of the safety of the open-look-see surgery of today, a later culture in which one has also had full opportunity. The second consideration is the careful choice of suitable subjects. The third is willingness to allow ample time for slowness of any given organ in its rhythm of contraction and relaxation, or for differing rates of travel of the wave of change through the various parts of a particular uterus. Herein the try-out of the Swedish pelvic massage of Thure Brandt taught us a special patience.[2]

For a *palpation research* on human ovulation the desirable factors seem to be the following:

a. The subject should present minimal interference by adipose tissue in the lower abdominal wall and the omentum.

b. The abdominal wall and pelvic floor should not be tense, as in athletic women.

c. The intestinal tone should be good, so that little gas is in the bowel (and the bowel recently emptied).

d. A varicosity of the broad ligament should be emptied by uplift of pelvis.

e. The ovaries should be mobile and thus readily brought within reach; as is sometimes the case after a retroversion has been reposited and edemas have subsided, the ligaments being relaxed.

f. The pelvic floor should yield freely to facilitate the reach up to the left ovary.

g. The uterus would better be of the type given to well-marked rhythms of contraction and relaxation, and fully displacable forward.

h. That patient will be of most value who has some midinterval symptom such as breast ache, show, mucous discharge, well-defined pain or localized discomfort, especially if tenderness in one or the other lower abdominal segment can be elicited by deep pressure by her own fingertips. Also, in the case of distant residence, if she is one who will keep careful records.

*Read in part before Section on Gynecology and Obstetrics, New York Academy of Medicine, April 26, 1932. From the National Committee on Maternal Health, Inc.

The time of day best for the individual may be found to be after breakfast and recent bowel action, and of course immediately after bladder emptying.

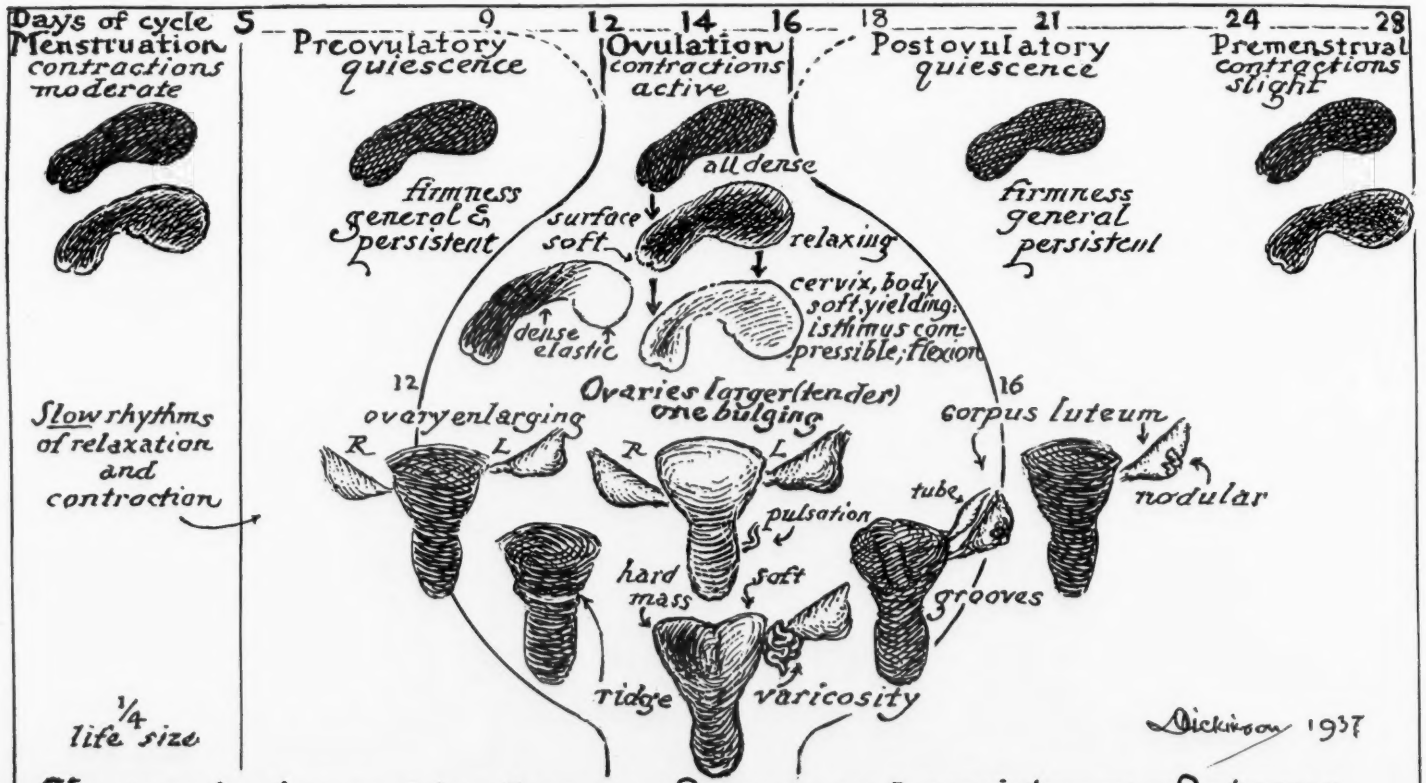
Posture is important. The tilting table that tips the trunk into a steep Trendelenburg may give what a level posture will not.[3] Full support of abducted knees relaxes circumpelvic tensions. The lower leg lifted level on Goepel troughs under calf and knee works better than the feet at the table level, provided the apparatus is comfortable. In some women with a cushion under the buttocks, full flexion of thigh on trunk gives high reach because of relaxed belly walls, while in some women the thighs press on the abdomen and increase tension. In other words, intelligent choice and individual adaptation may make the difference between an ovary completely defined and one quite elusive.

The substance of the previous study was as follows: It covered 89 observations on 5 patients chiefly during the interval. Both ovaries were found to show swelling and tenderness, but one much more than the other, even enlarging to twice the size. A *protruding rotundity* could be outlined in about half the instances, and a few days later, a nodular smaller lump, like one side of a blackberry. At the latter stage the tube on the corresponding side might be thickened.

At the time of unilateral ovarian enlargement the uterus in clear-cut examples showed a marked alteration in consistency, not unlike that in early pregnancy. Softening of the isthmus, the old Hegar sign, or my signs, the nodular feel of one upper angle, or any one of a variety of ridges or grooves might be present.

These grooves, lengthwise and transverse, on corpus, isthmus or fundus, and the nodular or cornual asymmetries[4] are confirmed by the x-ray shadows that depict clearly the striking alteration of the form of cavity of corpus and cervix during the waves of relaxation and contraction, general and local. These forms of cavity are carefully summarized in diagrams in Figs. 30 to 33 in *Human Sex Anatomy*. The chief change was *contraction and relaxation in a rhythm* running from 2 to 10 or even twenty minutes. During contraction any flexion, normal or marked, wholly or partly straightened out. A wave of contraction can start at the soft cervix and pass to isthmus and corpus. There can be complete softening of the whole organ except a core of cervix.

Before and after this rhythmic behavior in the midinterval, there are usually zones of complete quiescence which are almost diagnostic. During menstruation and preceding it, a minor capacity for these changes in consistence exists. In well-marked examples the difference in anteroposterior corpus diameter at the midinterval may be as much as one-third. Palpation of the uterus under favorable conditions



Changes in size and tenderness of ovary and consistency of uterus during the menstrual cycle: dark shading indicates degree of hardness

is easier than palpation of the ovaries, but it involves patience in all slow-acting musculatures. Erotic arousing does not seem to affect the phenomena. The much exploited rhythms in orgasm fall in the class of erotic fiction until evidence by trained observers is submitted.

Frequently in my histories the pronounced swelling and acute tenderness is labeled *ovaritis,* recurring ovaritis, even alternating ovaritis or salpingitis.

A new point is the vacillation of *varicosity* of the broad ligament with a pronounced unilateral finding at midinterval, and a change from side to side in sequent months. My form of examining table that allows a wide range of tilt permits emptying and filling of the vessels and confirmation of the diagnosis.[2]

For observation of the midinterval, conditions are favorable with gynecologists who give to the pessary a fair trial in those mobile retroversions which produce symptoms, then watch the results. They are especially favorable when teaching intelligent patients to do most of their own watching of position and pessary and ovarian tenderness. By self-diagnosis of relapse, by self-reposition and self-placement, the patient in many cases is enabled to leave the pessary out except when discomfort gives her notice, or the special relaxations and congestions of the period and of the midinterval are on, or when a long motor trip or a horseback ride calls for support, the pessary being used as needed, until pregnancies are behind her and the question of suspension calls for decision. In all these cases the ovulation period can be the time of election for special observation of the dependability of a "rhythm" and "safe period" for the particular individual.

The ovaries have been supposed to alternate in functioning, and this may be true, as Morse shows in rhesus monkeys.[4a] The higher position of the left ovary may account for the fact that in Case 1, tenderness at ten to twenty-three days was found on the left side nine times, on the right twice, in the center twice.

I was fortunate in being able to find, in a university neighborhood close to my residence, five intelligent women volunteers meeting the above specifications who, on the way to market after breakfast, stopped in about every second day, especially at the midinterval, for three periods. Gratitude and scientific interest on the part of wife and husband, together with the desire to estimate the safe period, brought this to pass. In research done in a clinic one would pay patients at the end of each ten or fourteen days, as our Committee did with the daily vaginal smears done at the Woman's Hospital by a technician under Dr. Papanicolaou.

As the pelvic findings of two of the cases have been pictured rather fully in my book entitled *Human Sex Anatomy* and as one of these cases

is repeated in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (November, 1936). I shall here turn to other cases, and dwell particularly on the relation between findings on examination and symptoms.[4a]

CASE 1.—Time of study, sixty-two months in sequence, with very good entries in diary for 50 of these. A mobile retroversion with severe dysmenorrhea in one who lived far away and declined operation, could be effectively self-diagnosed, self-reposited and choice of pessaries be self-placed, after due instruction, with relief of symptoms, long motoring and horseback becoming comfortable even during periods.

Examination, 12 observations:

One ovary large, one small, day of cycle	11	11			13	14		21					
Day before next period	20	24			17	22		14					
Corpus luteum, supposedly:							18						
Negative findings, ovaries, day of cycle 6			12				18			25	26	28	
Day before next period 28			21				17				10	2	

Thus four positives and six negatives may be said to point to the usually accepted time for ovulation, centering on the twelfth to fourteenth days (but with one finding of enlargement at the very last limit), and with a small grouping of erotic feeling thereabouts.

Tenderness as found by this Mittelschmerz patient 10 times:

Day of cycle	10	11	12	13	13	13	17	18	18	21	
											the average 14
Day before next period	14	18	21	17	15	19	8	11	14	10	
											the average 15

Spotting of blood, 9 reports:

Day of cycle	12	13	15	16	18	20	21	22	23	
										average 15.5
Day before next period	18	14	3	14	14	12	6	8	9	
										average 10

Because of the Ogino-Knaus teaching that the count should be taken, not in days beginning with the first day of the period, but backward from the first day of the succeeding period, the figures are given in both fashions above. The Knaus fourteenth day (and none other) and the tenth to eighteenth day subtraction show up 14 times in 19 observations of tenderness and spotting.

As between observations pointing toward the right or left ovary, bimanual examination showed enlargement of right, 5 times, of left, 2 times
Tenderness elicited by patient right, 2 times, left, 7 times
Together $\overline{7}$ times, $\overline{9}$ times

The right, being lower, is more accessible to a doctor's fingers: the left, lying higher, is more nearly within reach of a patient's pressure.

The *occurrences distributed day by day of the cycle* are as follows, using the letters "ex" for examination, "t" for tenderness and "b" for blood:

10	11	11	12	13	14	15	16	17	18	19	20	21	22	23
t	t	ex	b	ex	ex	y	b	t	t	b	b	ex	b	b
		ex		t	ex							t		
					ex							t		
					t							erotism		
					t									
					t									
					t									

[4a] Dickinson, R. L.: Am. J. Obst. & Gynec., November, 1936.

Thus putting together fifty months of record and 33 observations involving (a) bimanual examination of ovaries; (b) interval tenderness, self-observed; and (c) spotting, the *scattering is wide, involving every day of the cycle from the tenth to the twenty-third.* There is a grouping of enlarged ovary and tenderness in the expected space between the eleventh and the fourteenth days, 11 items; and a second minor grouping on the twenty-first day, 3 items; and with 4 spottings grouped hereabouts also. Among the 11 notes on tenderness, the averages run fourteen days after beginning of period, fifteen days before the start. Erotic rousing (without male), well marked or with self-relief, has 26 notes, scattered, with 10 after the period, and 7 near the twenty-first day.

There are all degrees of rhythmic contraction at ovulation time, apparently. The most complete example found in my short series was deliberately selected and used in both my previous publications in order to exhibit all the points. Here is an example on the opposite end of the scale, including a picturesque occurrence seen only once.

CASE 2.—At the twelfth day one ovary was found to be nearly double the size of its fellow. At the fourteenth day, with the two fingertips of the right hand easily holding the right ovary against the firm right pelvic wall, a small grape collapsed, and this ovary became about the size of its fellow. Two days later the area seemed nodular, but by no means as clearly so as in patients examined later in the cycle. The uterine contractions were very slow and not easy to elicit on these first two examinations, with twelve minutes to soften the isthmus and thicken the corpus, and 2 to 4 to harden and shrink. It is my guess that this condition was part of a generally defective tone of muscle and vessel. Though showing high fertility, fair menstrual regularity and strong sex desire, this lady presents general muscular flabbiness, while her uninjured pelvic floor all but sags. It was noted in removing a dermoid before her pregnancies that the pelvic tissues everywhere showed markedly relaxed vessels. Thus sluggish uterine musculature and venous stagnation might be called upon to explain torpor in rhythms, particularly lack of that striking alteration in thickness supposed to be largely due to filling and emptying of the layer of meshed vessels beneath the outer muscular layer which is clearly depicted by Bayer.[5]

Rouget,[6] in 1858, found the uterus thicker anteroposteriorly at menstruation and ovulation, but did not note the vacillations; he demonstrated that, postmortem, by complete filling of the vessels, one could double the size of the organ.

CASE 3.—This patient gave opportunity, in early pregnancies and while watching the pessary between pregnancies, to compare four findings of consistency of the uterus at the sixth week of gestation with the findings at seven midintervals. As the follicle grows very large in this patient, with the ovary sometimes as big as the fundus, it is favorable for comparison, twice as discovered on the left and three times on the right, with one temporary tubal thickening accompanying the ovarian enlargement of the same side.

Only once have I been able to hold a naked uterus in my hand (at laparotomy for suspension) and detect active contraction. It is presumably the anesthesia that arrests this activity, while the speed of our operative process defeats a watchful wait of ten minutes for observation. Monkey laparotomy might demonstrate the point.

Among my old histories I find 287, the first in 1886, in which the diagram and the accompanying notes show the uterus varying markedly from the usual consistency, ranging from that of the raw potato to or

toward that of the ripe tomato, in whole or in part, in the same person. The most frequent finding is the compressible isthmus, the next the thickened, rather soft or very elastic corpus, with the supravaginal cervix generally maintaining its rigid incompressibility. The grooves and the hard lump in a half-corpus are sometimes present. Unhappily, the exact day of the cycle is infrequently stated.

SUMMARY

Under specified favorable circumstances bimanual palpation in women can detect changes in the ovary and uterine contraction which point to the time of ovulation or omission of ovulation. The tenderness is often such as to suggest ovaritis or salpingitis. Varicosity of a broad ligament may be present only, or accented only, at the midinterval. In one patient with a five-year report, symptoms, Mittelschmerz, spotting and self-detected tenderness, were scattered from day ten to day twenty-three. In another the nonpregnant uterus of the midinterval copied her six weeks' pregnancy findings.

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RESECTION OF THE PRESACRAL NERVE IN THE TREATMENT OF OBSTINATE DYSMENORRHEA

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RESECTION of the presacral nerve, which I performed for the first time in December, 1924, is now regarded favorably by many surgeons. Judging from the literature on the subject, it may be said that it is now adopted in almost every country in the world. It seems, however, that while the operation is winning converts each year in France, in foreign countries its use is still confined to a small minority. The number of women, therefore, who are able to benefit from this procedure, remains still limited. After an experience of twelve years with the operation, I may say that I am more and more convinced of the value of resection of the presacral nerve in every syndrome associated with an anatomic or functional disturbance of the hypogastric plexus, and that I know of no other therapeutic method that may be substituted for it. Adson, one of the last converts to presacral sympathectomy, declares too, in a recent article, that, after having observed the results obtained, the operation seems to him much more valuable than it did at first. Indeed, of all the surgeons who have performed presacral resection correctly in cases in which the operation was indicated, I do not know one who abandoned it later. In general, it may be said that, whenever the indications were properly observed and the operation correctly performed, the results conformed to those I described in my first communications.

I shall not, at this time, discuss either the technic or the indications for the operation, but refer those interested to the various reports I presented on this subject, in particular to the general study made in my book *Chirurgie du Sympathique pelvien en Gynecologie*[1] or in the *Journal International de Chirurgie*.[2] I intend to confine myself here to a discussion of some ideas on the treatment of severe functional dysmenorrhea, which is one of the most common indications for resection of the presacral nerve.

Normally, in most women, menstruation is unaccompanied by painful sensations. The evolution and maturation of the graafian follicle and its rupture which, in the majority of cases, precedes the thickening of the uterine mucosa in preparation for the embedding of the ovum, as well as its destruction, in the absence of fertilization, cause neither serious discomfort nor pain. The autonomic nervous system seems to remain indifferent to the biologic phenomena, marking the different stages of the menstrual cycle. Why then, are all these physiologic

phenomena characterized in certain women by pains that necessitate rest in bed, cessation of work, and the use of narcotics? Under what influence does the autonomic nervous system, which remains in some way impassive in the course of repeated cycles, acquire such a hyperirritability as to cause severe pain associated with the preparation and shedding of the endometrium?

Former gynecologists attempted to explain this fact by the existence of a cervical stenosis or of an endometritis which they treated by dilatation and curettage. Simple explanations appeal to us most, which may account for the favor accorded this theory until recently.

However, one has but to examine the facts more closely to realize that usually at the onset of functional dysmenorrhea, there is neither endometritis nor a stenosis of the cervix or of the isthmus. Doubtless, before we were acquainted with the evolution of the uterine mucosa, the premenstrual hyperplasia observed in cases in which a curettage had been performed, might have been taken for an inflammatory lesion of the endometrium, but we know today that the latter is in reality dependent on the ovarian hormones. And the best proof that inflammation does not seem to play a great rôle in the occurrence of dysmenorrhea, is that most cases of adnexitis develop without ever causing severe or stubborn dysmenorrhea.

The arguments that can be advanced against a mechanical cause of menstrual pains are still more numerous. Without discussing here the observations of some gynecologists who have sought in vain for a stenosis or a spasm of the uterine isthmus, it is an irrefutable fact that severe, stubborn dysmenorrhea which requires surgical intervention is almost always secondary in origin.

Actually, in most cases, we deal with girls who menstruated normally for one, two, three, or four years and sometimes longer and then began to experience such menstrual pain that they were forced to take to bed. Sometimes this occurs after acute serofibrinous pleurisy that developed without complications, or after erythema nodosum, or any other attenuated tuberculous manifestations without there having been any localization of tuberculous lesions in the ovary or the uterus. But generally, the most careful inquiry into the history reveals no cause for the occurrence of the dysmenorrhea. If we add to this the fact that the most thorough dilatation, and even parturition, is not always sufficient to cure menstrual pains, then one is forced to give up the idea of stenosis as a causative factor. I am well aware of the fact that menstrual pains often assume the character of true uterine colics and that in about half the cases dilatation of the cervix causes them to disappear. One, however, is not justified in assuming a relationship between the stenosis and the dysmenorrhea, for one may obtain with dilatation of the anus as good or better results. It is highly probable that the method works by stretching of the very

numerous sympathetic fibers in the cervix and the uterosacral ligaments. Last, I may add that the concept of stenosis could not explain "painful amenorrhea" to which Dalche has drawn our attention, and which is characterized by painful periodic attacks at the date when menstruation should take place, even when there is no serous or bloody discharge from the uterus. I have, on several occasions, operated upon such patients in whom dysmenorrhea alternated with painful amenorrhea. I found no lesions of the genital tract that could explain the phenomenon.

In view of these facts, and the frequent failures following dilatation and curettage, I was led to seek another form of therapy. Having had the opportunity of observing a certain number of patients in whom the dysmenorrhea was associated with other complaints indicating a dysfunction of the sympathetic nervous system, e.g., dyspareunia, vaginismus, cystalgia, sensation of burning on urination, tenesmus ani, vaginal pain, pruritus vulvae, sexual hyperexcitability, I was led to do, at first, periarterial sympathectomies of the hypogastric arteries, and later, resection of the presacral nerve.

Former authors would explain all these phenomena collectively or separately, on the basis of a neurosis, anxiety-neurosis, neurarthritis, spasmophilia or hysteria. Today, they are generally attributed to a dysfunction of the autonomic nervous system, the abnormal hyperexcitation or hyperexcitability of which is responsible for the symptoms enumerated above. According to this concept very slight injuries of the genital organs and even remote pelvic lesions may sometimes provoke, either by direct irritation or by reflex action, sensory-motor or vasomotor disturbances in the pelvis, and cause pelvic neuralgia, dysmenorrhea or cystalgia, etc. Furthermore a very slight anatomic injury of an ovary, or of the endocrine glands connected with it, and even a simple disturbance in the evolution of the graafian follicle (the hormones of which exert an influence not only on the uterine mucosa and uterine contractions but also on the vagosympathetic nervous system itself) initiate, by reflex and endocrine stimuli, vicious circles which continue to persist until they can be interrupted. Last, it enables us to understand how a primary disturbance of the pelvic sympathetic system (hypogastric plexialgia or plexitis) or of the nervous centers connected with it, can lead to the same results.

Doubtless, this theory of the pathogenesis of dysmenorrhea is not immune from criticism, but it is justified by the fact that, while dysmenorrhea and pelvic neuralgia do not respond to the most varied operations on the ovary or the uterus, these conditions are generally completely cured by properly performed presacral sympathectomy.

As regards so-called essential dysmenorrhea, of nearly 300 patients upon whom I operated in the course of twelve years, I know of only two that were not relieved.

In the first case the patient was sympathectomized elsewhere by an excellent surgeon, but no doubt the resection had not been complete, and he had resected only the left part of the nerve, for I found on this side a cicatricial neuroma, the histologic picture of which I presented in my book. After the operation, the patient, who had been suffering continually from the neuroma, was at once relieved. Some months afterward, however, the periods again became painful. This led the first surgeon to castrate the patient.

The second case is more interesting. It was a girl, aged twenty-four years, who for five years had suffered from dysmenorrhea, refractory to all medical measures. In addition, for three years, the patient experienced intense sexual hyperexcitation, nymphomania and pruritus vulvae not explained by any local or constitutional cause. She was operated upon in 1934 and pruritus vulvae and sexual hyperexcitation disappeared completely. Unfortunately, the dysmenorrhea was not influenced. The patient continued to suffer at the time of her periods and I had to do a temporary x-ray castration.

During the laparotomy in this case, I had found signs of peritonitis in the culdesac with adhesions between the rectum and the posterior wall of the vagina, and some free peritoneal fluid. Can one ascribe the persistence of the painful periods to these findings? I do not think so, since if the local inflammatory process had been the causative factor, it seems probable that the pruritus vulvae and sexual hyperexcitation would not have disappeared. It is possible that the inflammatory reaction was due to an endometriosis that escaped me. In any case one cannot claim that the presacral nerve had not been removed or only incompletely, for resection had been particularly easy in that case.

Besides these unsuccessful results, three or four other patients still suffer during their periods, but only during one or two hours, instead of twenty-four or forty-eight hours, and in no case are they forced to stay in bed or stop work. While these were not completely successful, the results, however, were highly satisfactory to the patients.

In contrast to the few cases which were not as fully relieved as I had expected, resection of the presacral nerve led to a complete cure in all the other cases. It is impossible to present here, even in abstract form, all the facts relevant to these cases. I will confine my remarks to some of them that were particularly striking.

The following case affords an example: A girl, aged twenty-two years, menstruated from the age of fourteen. Her periods were at first normal, later on they were accompanied by more and more severe pains, which finally impaired her health. As a child, the patient had had a bronchitis which resulted in pulmonary sclerosis. For this reason, during six years, since she could not bear the climate of her birthplace by the side of the Loire, she led a rather unhappy life in various preventoria. One day the Director of the sanatorium where she was being treated, seeing how severely she suffered during her menstrual periods, sent her to me. As the pulmonary lesions were not active, I proposed operation. Her father was very unwilling to agree to this since two years before she had had an appendectomy for the dysmenorrhea without any relief. But the operation seemed to me all the more necessary since for some months the patient had also developed pain in the vagina and nymphomania. Resection of the presacral nerve performed five years

ago relieved all her symptoms. The patient gained nine pounds in three months after the operation and seventeen pounds during the ensuing year. After having remained six years away from home, she was easily acclimated to her birthplace which she has never left since. Quite recently she wrote me, "It is to your operation, dear Doctor, that I owe my return to my family and my active life, there. I shall always be very grateful to you." Since at the time of the operation I could discover no pelvic lesion, it was obviously a case of functional dysmenorrhea.

Another case, a girl aged twenty-eight years, was under treatment in the mountains, for very serious pulmonary lesions and a cavity of the right lung. After fifteen or eighteen months, as the pulmonary condition began to improve, the patient, whose menstrual periods had never been painful, began to suffer from dysmenorrhea so severe that during the week of her flow she rapidly lost the two or three pounds which she had gained in the preceding three weeks. When I saw her for the first time, she was not yet in good enough general condition to allow me to operate. I stated, however, that the occurrence of dysmenorrhea should be considered as a favorable prognostic sign. I have never seen dysmenorrhea appear this late in the course of tuberculous disease which went on to a fatal outcome. Some months afterward, the pulmonary lesions being no longer active, I resected the presacral nerve. From that time the cure proceeded rapidly. The patient, who weighed only 80 pounds, now weighs 130 pounds, and she no longer suffers during her periods.

In passing, I may mention that there were a number of cases of young girls who had had an appendectomy to relieve the dysmenorrhea. These, in the face of the steadily increasing pain, consented to a second intervention, though not without reluctance, because of the disappointing results of the appendectomy which they felt, moreover, had aggravated the dysmenorrhea. This second operation brought a complete and definite cure to the dysmenorrhea. Many of them married and are now very happy mothers.

It is not only from a pathologic point of view that resection of the presacral nerve has a good effect in relieving dysmenorrhea. In another article, I described the case of a girl, the sister of a surgeon, who was obliged every month to remain in bed at the time of her periods. Two years after the operation (which her brother had long hesitated to advise, always hoping the condition would finally subside) she wrote to thank me. Had she not been operated upon, she would not have passed her examinations as a nurse, for on the examination day she would have had to remain in bed. She was operated upon four years ago and was completely relieved of her pains. She now leads an active life as a district nurse without ever being incapacitated.

I also quoted the case of a girl, aged nineteen years, the daughter of a well-known physician. Her dysmenorrhea began after acute serofibrinous pleurisy that developed without complications, and she had such painful paroxysms that for forty-eight hours she was obliged to stay in bed and was not relieved by any drug including belladonna, papaverine, and all the opiates used in such cases. She would vomit a great deal; the temperature during the forty-eight hours would remain at 40° C. and would return to normal at the end of the painful period. Several times, this girl, who was very athletic, had stopped during the winter in the mountains and while there she had suffered from attacks of painful amenorrhea. Professor Maranon, when consulted, had attributed her symptoms to a dysfunction of the

anterior lobe of the pituitary gland and prescribed treatment with prolan. This form of therapy was ineffectual. I was then called in consultation and resected her presacral nerve without any additional surgical procedures. Following this her symptoms disappeared. She was operated upon two and one-half years ago and she has not suffered during her menstrual periods since then. She was married last year and has now been pregnant for four months.

These few instances, recalled from about 300, are typical. One can best judge the value of the operation by the results obtained in cases of membranous dysmenorrhea. The refractory character of this type of dysmenorrhea is well known. The pain experienced, together with the expulsion of fragments of a thickened mucosa (menstrual decidua), occasionally simulates a miscarriage. I operated upon a patient who in spite of two previous deliveries still suffered during her menstrual periods. She was operated upon five years ago. Since then she has had no complaints, although she still expels large pieces of mucosa during her periods. She had another normal pregnancy and an interstitial pregnancy for which Convert, of Bourg, performed a conservative operation.

In another case (my youngest patient) a girl, aged fourteen years, menstruated from the age of ten. Her dysmenorrhea (membranous) was so painful that at thirteen she was obliged to leave school. Here again, resection of the presacral nerve, by curing the dysmenorrhea, enabled her to continue her studies, to pass her examinations, and become a chemist.

I could cite many other interesting cases. It is sufficient to say that in the treatment of severe dysmenorrhea, no other method has ever given such consistently good results.

It is a popular belief that marriage will frequently relieve dysmenorrhea, which is indeed true in many cases in which the menstrual pains are easily controlled with the usual sedatives and which are not severe enough to incapacitate the individual. However, in severe dysmenorrhea not relieved by opium, belladonna and such opiates, which necessitates rest in bed and is accompanied by sympathetic disturbances, conjugal intercourse does not relieve the condition and the coital act is frequently difficult and painful and only adds one more tribulation to the life of these patients. I believe, therefore, that it is preferable to operate upon them without exposing them to this distressing trial.

I wish to emphasize that resection of the pelvic sympathetic nerve is suitable only in cases in which menstrual pains are not caused either by malformations or adenomyomas of the cornua or endometriosis of the ovaries, of the tubes, or of the rectovaginal septum, or any other still more evident lesion of the genital organs. It is valueless for pain of ovarian origin or intermenstrual pain which is associated with ovulation. This type of pain is generally not influenced by resection of the hypogastric plexus. This is to be expected since the ovaries receive their nerve supply from another pathway through the utero-

ovarian nerves. This is so striking that we sometimes see patients with both menstrual and intermenstrual pains, in whom following resection of the presacral nerve, ovulation alone remained painful, the menstrual periods causing no discomfort. In these cases, complementary resection of the ovarian nerves is sometimes necessary. Resection of the lumbo-ovarian nerves, or the "isophenolization" of the uteroovarian plexus has not always, in my experience, given satisfactory results.

I wish to point out that in a number of the cases reported in the literature as unsuccessful, failure has been due either to incomplete resection of the nerve or to the fact that the operation was not indicated in the cases chosen.

The mortality rate is that of all simple aseptic abdominal operations, about 1 per 100. In more than 300 operations I lost only two patients who died from acute pulmonary complications. I never noted any abdominal complication immediately or subsequently, and I never observed trouble with the sphincters or with the genital organs. More than 50 patients have had consecutive pregnancies and no accident was ever noted during parturition.

In the absence of precise and certain physiologic data concerning the nature and origin of the constituents of the presacral nerve (superior hypogastric plexus of Hovelacque), it is difficult to explain the successful results of presacral sympathectomy. Is it due to the suppression and interruption of abnormal sensory-motor reflexes or modification of the pelvic circulatory system? Both theories can be postulated. Adson states that dysmenorrhea as well as the other genital disturbances for which operation is required, are produced by abnormal stimuli from superior autonomic nervous centers of the midbrain which are connected with the pituitary gland. Resection of the presacral nerve is effective because it suppresses the connections of the genital organs with the nervous centers. However hypothetical those theories may be, it is quite certain that in the syndromes of hypogastric plexalgia, and particularly in dysmenorrhea, which is the most frequent manifestation of its dysfunction, well-performed pelvic sympathectomy yields uniformly good results. Accordingly, when all of the known therapeutic measures have been ineffective, it seems wisest to advise early operation. Furthermore, there is the possibility that a slight anatomic lesion (ovarian endometriosis, adenomyosis of the cornua for instance) which had not been discovered by clinical examination may be found, treatment of which will be sufficient to relieve the dysmenorrhea. But in the other cases, resection of the presacral nerve will effect a cure in patients who have been refractory to all other forms of therapy.

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THE VALUE OF URETERAL IMPLANTATION INTO THE BLADDER AND INTO THE SIGMOID IN GYNECOLOGIC INJURIES AND DISEASES

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FORTY-THREE years ago, Florian Krug of New York stated that "accidental injury to the ureters is one of the most unfortunate occurrences of abdominal surgery . . . the almost universal adoption of the Trendelenburg posture has greatly reduced the liability to this accident, as it enables us to operate under the guidance of the eye as well as of the touch. Still, there are cases, and always will be cases, where, owing to the nature of the disease, or some anomaly of the relative position of the ureter, injury to this organ is unavoidable."

In case of such injury, if detected during the operation, naturally an immediate reimplantation of the ureter into the bladder should be done, if possible. These cases present a group with which the gynecologist has to deal immediately, and are rarely seen by the urologic surgeon. The names of Krug, Baldy, Stoeckel, Franz, Kroenig, and Sampson are intimately connected with the development of this branch of surgery. Not only did they have the opportunity to perform an immediate repair to the damaged condition, but often, after recuperation from the first abdominal procedure, they had the opportunity to correct the fistulous leak resulting from damage to one or both ureters.

In the following brief paper, I shall refer to the three types of gynecologic patients, who from time to time seek urologic surgery for the relief of disturbances in the ureter, caused by operative interference in the pelvis by the gynecologist, by disease in the pelvis (parametritis), as well as those cases of incurable vesicovaginal fistula, which are, despite numerous attempts at closure, passing all their urine through the fistulous opening.

Through close association with the gynecologists, one is able, perhaps, to see more of these cases than is the lot of most urologic surgeons. The cases about to be reported are grouped as follows:

Group I.—Cases of compression stricture of the ureter due to parametritis and periureteritis, in which neostomy is necessary to relieve the painful condition in the kidney and get adequate drainage of the obstructed hydroureteronephrosis.

Group II.—Cases of ureterovaginal fistula, caused by damage to the ureter in either pelvic or abdominal procedures.

Group III.—Cases of large, incurable, vesicovaginal fistula, in which the ureters have to be implanted into the sigmoid, to deflect the stream of urine.

Prior to the introduction of excretory urography in 1929, it was impossible to follow these cases postoperatively intelligently and determine how well the reimplanted kidney functioned. Naturally, it was impossible to control the output in the upper urinary tract after ureterosigmoid anastomosis. Occasionally with the ureterovesical anastomoses, one should catheterize the reimplanted ureter and de-

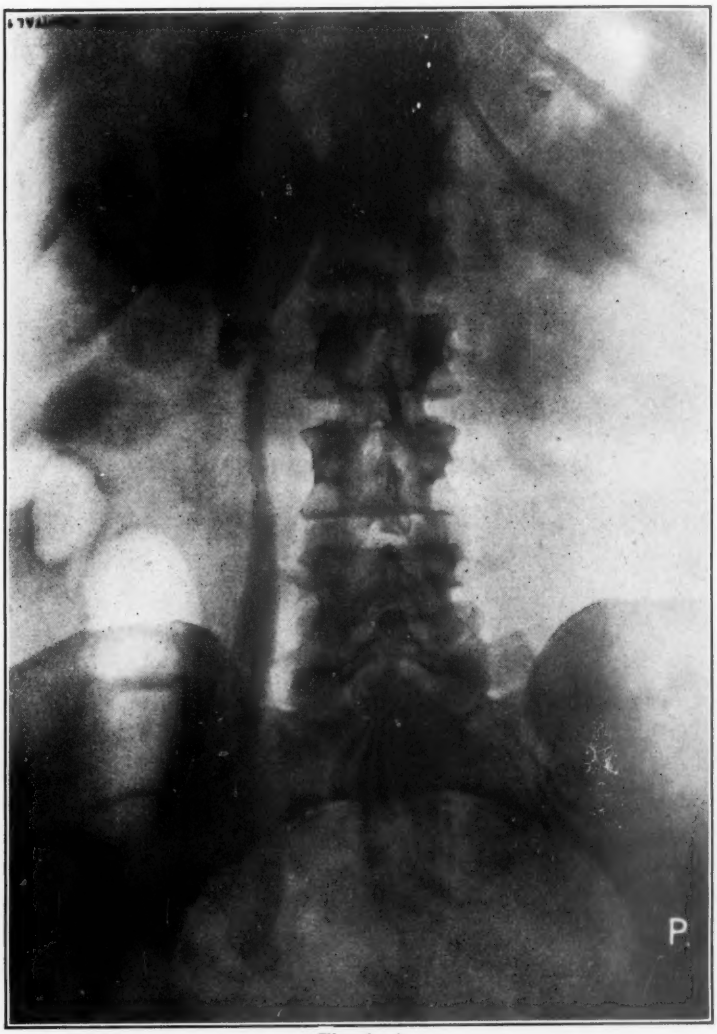

Fig. 1, *A*.

Fig. 1.—Case 1, P. L., aged fifty years. *A*, Retrograde pyeloureterogram before operation, showing dilated pelvis and obstructed lower ureter, ending in bulb. Picture taken seven hours after injection of iodide solution. *B*, Excretory urogram before operation, showing practically same condition with bulbous lower end of right ureter. *C*, Excretory urogram four-plus weeks after ureter neostomy, showing right kidney pelvis much smaller and ureter filling all the way down to bladder, only moderately enlarged. *D*, Excretory urogram almost five years after right ureter neostomy, showing marked reduction of the hydronephrosis, practically normal ureter throughout.

termine, more or less satisfactorily, the function of the reimplanted organ. In this field, however, excretory urography has proved of inestimable value, as it not only gives the picture of the anatomical

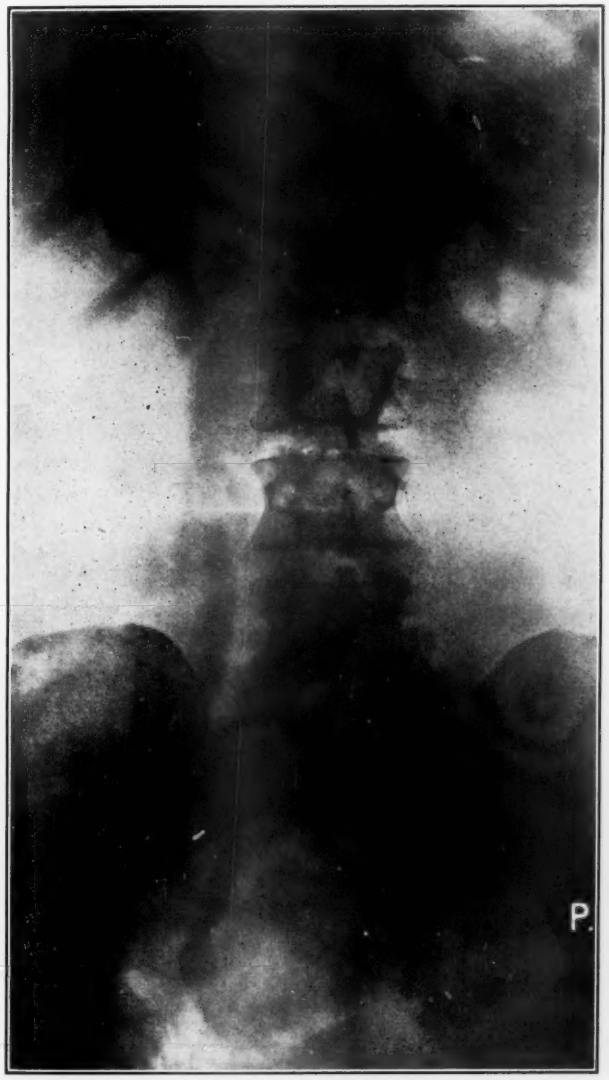

Fig. 1, *B*.

condition of the ureter and bladder, but at the same time gives a fair indication of the relative function of the reimplanted organ.

To illustrate these three types of cases, I have selected a series of three cases, that demonstrate rather conclusively the great value of conservative surgery under these conditions. The urograms are par-

ticularly instructive and, in some cases which have been followed for years after the ureteral transplantation, the roentgen studies are particularly illuminating.

Fig. 1, *C*.

CASE 1.—*Obstruction of the right ureter by periureteritis (parametritis), producing hydroureteronephrosis.* P. L., aged fifty years. Retrograde urogram showed extensive dilatation of both ureters and kidney pelves. Patient had recurrent obstructive symptoms on the right side, and the retrograde-filled right kidney did

not empty completely after seven hours. The lower end of the ureter was bulbous and dilated and remained so, despite stretchings continued over months. Excretory urogram showed the same ureterohydronephrosis with stricture in the right ureter close to the bladder. Jan. 5, 1932, right neostomy, extraperitoneal. Stricture found behind uterine vessels. Considerable periureteritis. Indwelling urethral catheter. Considerable urinary leakage from wound, as if ureter had pulled out. Tension

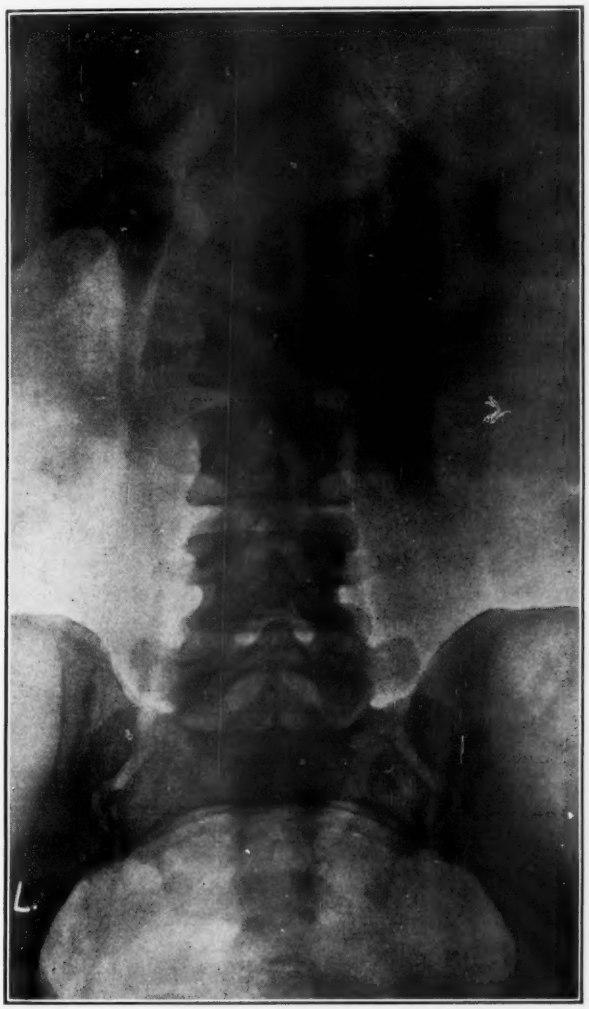

Fig. 1, *D*.

had been considerable at operation, the large thick ureter being freed retroperitoneally well into the lumbar gutter, and the bladder was mobilized as well. Jan. 25, 1932, cystoscopy, right ureter easily catheterized. Feb. 11, 1932, excretory urogram showed good function both sides. Feb. 27, 1932, chills, fever, frequency and right kidney pain and tenderness. Cystogram in Trendelenburg position showed no reflux. Symptoms subsided and on April 20, 1932, excretory urogram

showed right kidney emptied well and the ureterohydronephrosis was less marked Grade 1—plus. Sept. 10, 1932, cystoscopy, good indigo carmine at fifteen minutes from right kidney, No. 5 ureteral catheter passed to kidney pelvis and 15 c.c. clear urine aspirated. Dec. 4, 1936, excretory urogram showed practically normal ureter throughout, marked reduction of the hydronephrosis (Fig. 1, *A-D*).

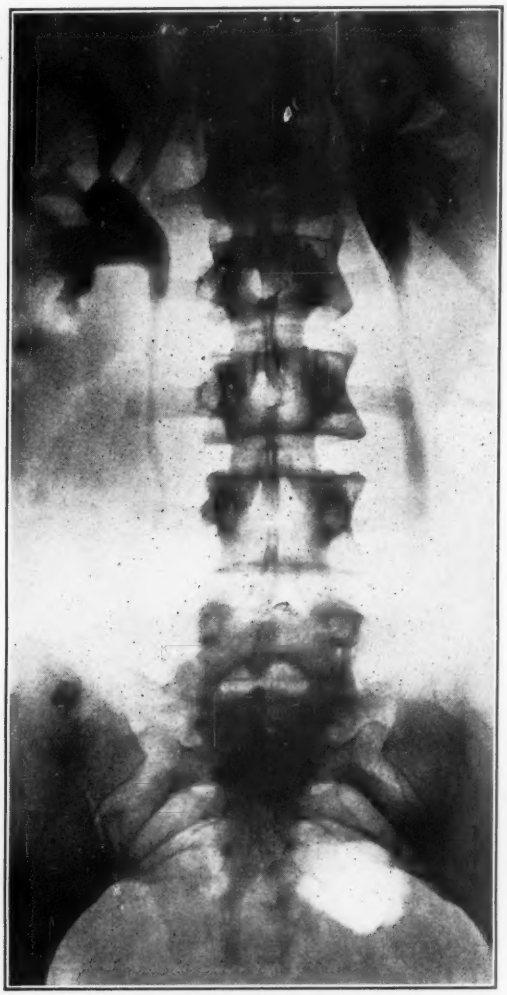

Fig. 2, *A*.

Fig. 2.—Case 2, H. M., aged twenty-three years. *A*, Excretory urogram ten months after left ureter neostomy, showing normal upper urinary tract. *B*, Excretory urogram two and three-fourths years after left ureter neostomy, showing ureters in pelvis absolutely normal in caliber, and on left side whole ureter is filled, as well as kidney, and calices showing normal contour. Impossible to distinguish between two sides as to which has been operated upon. *C*, Excretory urogram seven and three-fourths years after left ureter neostomy, showing good function both kidneys, ureter can be traced down to bladder, showing one dilated spindle over the sacroiliac joint, and another one just external to the bladder.

CASE 2.—*Ureterovaginal fistula following gynecologic operative injury.* H. C. M., aged twenty-three years. October, 1928, gynecologic transvaginal procedure, and ten days later vaginal urinary leakage. March 8, 1929, left ureter neostomy, extraperitoneal route. Bladder drained with a transurethral Pezzer catheter.

April 29, 1929, cystoscopy, left ureter catheterized, no indigo carmine at twenty-five minutes (intramuscular injection), specimen clear. Right kidney strong indigo carmine. Jan. 27, 1930, excretory urogram showed normal left ureter and kidney. Right pelvis slightly dilated. Dec. 8, 1931, excretory urogram showed absolutely normal pictures in both kidneys and ureters. Dec. 8, 1936, excretory

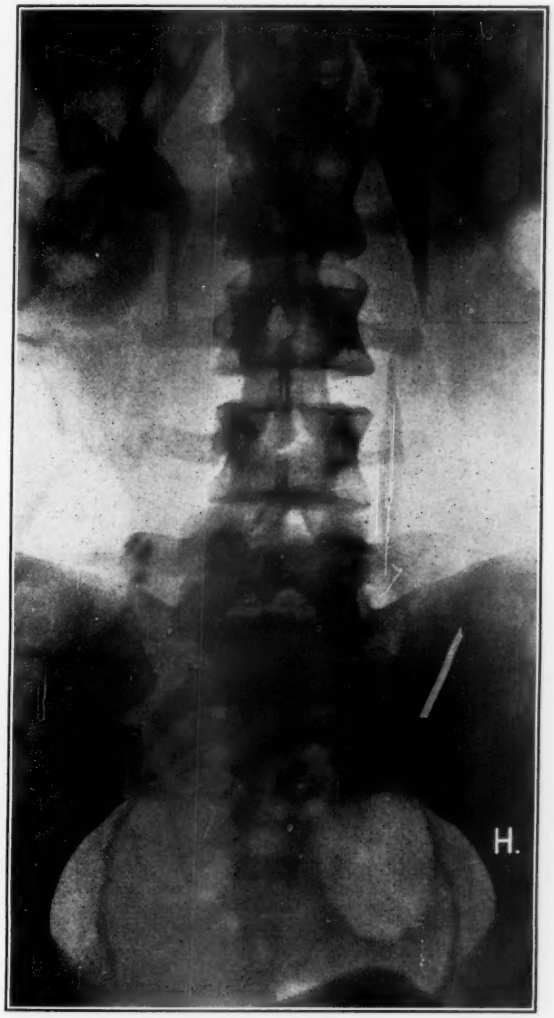

Fig. 2, *B*.

urogram showed normal left kidney, good function, and ureter can be traced down to the bladder, showing one dilated spindle over the sacroiliac joint and another one just external to the bladder (Fig. 2, *A-C*).

Case 3.—*Vesicovaginal fistula, following cesarean operation.* G. S., aged thirty-six. In 1932, following forceps and cesarean operation, developed vaginal fistula, complicated by calculus in the bladder after the first attempt to close the fistula. The calculus formed about a foreign body. In all there were eight attempts at

repair, without success, so that in 1936 the patient was leaking vaginally and losing all her urine. Excretory urogram showed good function, normal anatomy in the upper urinary tract. Blood urea normal. After preoperative preparation with castor oil, colon irrigations, followed by constipating diet, on Feb. 4, 1936, a left ureterosigmoidostomy was carried out. By the ninth day following the implantation of the ureter, there was copious output of urine by rectum and before the

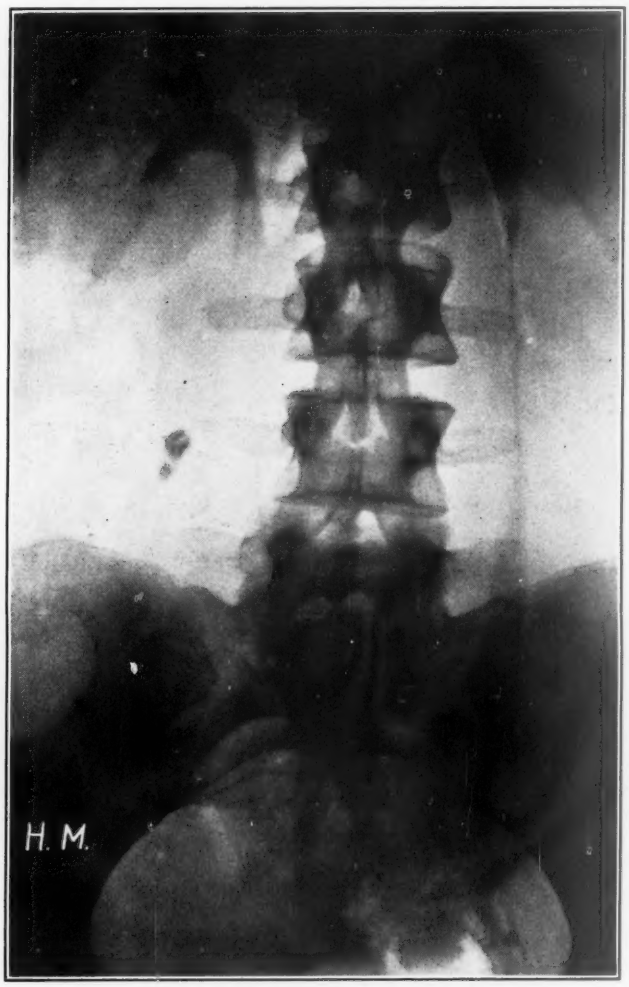

Fig. 2, *C*.

patient was discharged from the hospital, she was passing fluid movements, some of which were almost pure urine, four or five times a day with perfect control of her rectal reservoir. Excretory urogram was made on the twentieth day following the implantation of the left ureter, and showed a moderate left hydroureteronephrosis. On April 14, 1936, as the patient had no symptoms from the left ureter anastomosis, a similar anastomosis was made between the right ureter and sigmoid. Patient stood the operation very well, there was no serious reaction, and she made an uneventful recovery. At both of these operations, the split end of the ureter

was drawn with a mattress stitch of Pagenstecher into the lumen of the sigmoid, after incising the serosa and muscularis down to the submucosa and making a trough in the wall of the bowel by pushing these structures aside and burying the ureter with two layers of fine chromic gut, Lembert sutures. Pezzer catheter was left in the rectum. June 20, 1936, postoperative excretory urogram showed ex-

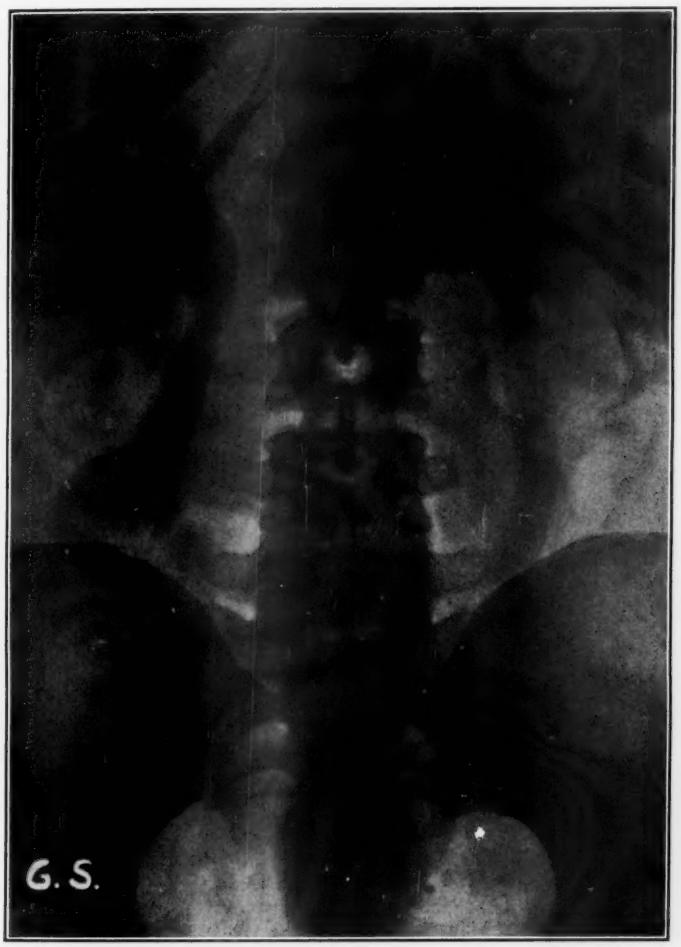

Fig. 3.—Case 3, G. S., aged thirty-six years. *A*, Excretory urogram ten months following left ureterosigmoidostomy, eight months following right ureterosigmoidostomy, showing moderate hydroureteronephrosis.

cellent function of both kidneys. There was slight dilatation of the upper urinary tract, as one sees so regularly after anastomosis of ureter and sigmoid. This dilatation may gradually disappear and normal anatomy be reestablished. The patient has had no attacks of pyelitis, and rectal control is perfectly satisfactory. Excretory urogram on Dec. 12, 1936, showed moderate hydroureteronephrosis (Fig. 3).

THE PATHOLOGY AND TREATMENT OF INFLAMMATORY DISEASES OF THE CERVIX

"The Pelvic Tonsil"

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THE cervix uteri is a halfway "stop" between two important highways. It is frequently the repository of the defects and weakness of both of these. It is influenced by the physiology and pathology of both of these avenues of approach. It is a barrier to infection, often suffering much in its defense of the sanctum uterinum. Unfortunately, it is situated in the very middle of the "silent area" of the pelvis—that territory bounded above by the peritoneum and below by the perineum, in which gross and grave pathology can, and does, exist without causing symptoms. This area, being part of the visceral field of the body, is almost devoid of tactile nerve endings (just as other intracorporeal viscera) and therefore pain—that announcer of physical ills—is quiescent in the pelvis, and disease may take hold and advance to incurable proportions before the victim is aware of its presence. The painlessness of cervical disease is proverbial. The delicate cervical columnar lining, set upon a functionally unchanging substratum of fibromuscular tissue, is in contrast to the ever changing uterine mucosa which sheds most of its sins of defect every month and renews its vitality with pregnancies. The cervical glands retain the surface scars of battle, the uterine mucosa casts them off—even the major scars of pregnancy. The cervical glands, racemose and deeply burrowing, become harbingers of infection by becoming retention cysts, and inflammatory disease of the fibromuscular tissue influences the superimposed protective columnar or squamous cells, producing in these either hyperfunction or hyperplasia, or both, and, if the process is more destructive, we find a loss of these protective surface epithelia and the initiation of erosions. Long-continued inflammatory irritation may at any time change controlled hyperplasia into uncontrolled invasion which spells cancer.

To understand the pathology of inflammatory diseases of the cervix and the consequences which follow from these, one must know the minute histology and physiology of this organ.

Histology and Physiology of the Cervix

Unlike the uterus, the cervix has no highly specialized intermediate cellular structure such as that in which the endometrial glands are

always imbedded. The racemose, deeply burrowing, cervical glands are imbedded in common clay, an ordinary fibromuscular tissue, differing in no particular manner from similar structures in other parts of the body. This fibromuscular tissue has several functions to perform. It acts as a nucleus into which the supporting tissues of the pelvic floor find an anchorage, thereby allowing freedom of movement to the superimposed uterine body. Movement to this portion is essential to the proper performance of its highest function. Second, the cervical fibromuscular tissues act as a sphincter to the uterine contents. To permit wide dilatation of this sphincter without undue destruction, the muscular element must greatly outweigh the fibrous element, thereby permitting of an elasticity which would be otherwise impossible. This normal proportion of muscular (sphincteric) and fibrous (supportive) tissues is frequently altered, always to the detriment of the muscular element, by errors in development, abnormal puerperiums, infection, newgrowths and age.

Musculofibrous tissue of the cervix differs in its pathology in no particular sense from similar tissues elsewhere in the body, except owing to the presence of two factors: the influences brought to bear by being invaded by glands, and the influences of the functional phases associated with procreation.

The glands of the cervix are a protective mechanism; they are constituted by digitations of a common duct lined by a single layer of tall columnar cells. These are mucous goblet cells, in which normally the tall cell has a nucleus near the base and an open end toward the lumen, like the cup of a tulip. Below these is a layer of flattened cells constituting the so-called basement membrane. Normally, the quantity of secretion is just sufficient to fill the cervix with a tenacious stringy plug which protects the uterine cavity from invasion. It flows slowly like a glacier. This plug liquefies, when in contact with the acid secretion of the vagina, and acts further as a lubricant to this canal. The reason for the digitations of the cervical glands is found in the desire to increase the extent of functioning surface and thereby lessen the function of each individual cell. It is a maximum of function with a minimum of exposed surface. Unlike the endometrium, the cervical tissue has a permanency which may act to its own detriment. The uterine mucosa, by shedding its surface, also sheds many of its diseases, as will be pointed out in another paper by me. The cervix retains the scars which it cannot heal by the ordinary corporeal reparative processes. The processes associated with procreation come but slightly, if at all, to its aid. The cervix undergoes slightly transitional changes with menstruation, and marked hypertrophy, increased vascularity and glandular function during pregnancy, with regression during the normal puerperium, but it has not the happy faculty of casting off the scars of this maelstrom

of cellular disturbance. It must depend upon the general recuperative properties of the body as a whole for its reversio ad integrum. The tissues of the genital tract suffer from a state of unrest, a flow and ebb, that may affect other parts of the body, but to a much less degree. This state of flux may operate to the advantage, or disadvantage, of the affected tissues. But it is often productive of pathologic states, or it may greatly modify these when they are present.

Inflammatory changes of the cervix may be simple or special, acute or chronic, or they may spend themselves chiefly upon the cervical glandular structures affecting the fibromuscular layers minimally, or they may affect these component tissues in the reverse order.

General.—Most inflammatory cervical diseases begin as an acute process and degenerate into chronic catarrhal states. Others are acute and short-lived. It is characteristic of acute mucous membrane diseases that, when they end abruptly, they leave a minimum of change in tissue or function. Chronic inflammatory disease, on the other hand, is productive or destructive, according to its intensity relative to the body reactions. Productive inflammatory diseases, that is, the more chronic processes, express their production in either hyperfunction or hyperplasia. The destructive types produce loss of tissue, expressed as erosions or ulcerations.

There are other functions which initiate productive changes in the cervix, essentially causing enlargement of the fibromuscular tissues, chiefly with minor incidental, or major accidental, changes in the endocervical epithelium. These are the cervical hypertrophies of pregnancy without normal retrogressive changes in the puerperium, leaving a permanently enlarged cervix usually associated with cystic disease. Other productive cervical diseases may arise out of the stimulus of corporeal fibroids. These, of course, simulate a pregnancy, causing hyperplasia of all the tissues as in pregnancy. After all, fibroids are the barren woman's children. When the uterus has prepared the functional endometrial and allied changes repeatedly without issue, fibroids develop as a pseudopregnancy. The influence upon the cervix is frequently quite similar. Such hypertrophic changes when not followed by retrogression, as in the normal puerperium, result eventually in a large, hard cervix, which, when associated with cystic disease, may assume large proportions. Since this is most commonly the aftermath of an abnormal puerperium, the cause, or causes which inhibited the reduction usually also affect the uterus, causing it to remain in a state of chronic subinvolution, thereby remaining globular, tender, large, superimposed upon a cervix similarly affected. In fact, the condition of the cervix is being recognized more and more as the index of the state of the uterus, and this applies as much to the mucosal as to the fibromuscular changes. Such conditions of the cervix should be distinguished by the name of "chronic interstitial

cervicitis," as contrasted with purely catarrhal endocervicitis, in which the disease is restricted wholly, or chiefly, to the glandular structures.

Acute Catarrhal Endocervicitis.—Acute catarrhal endocervicitis may arise from any acute infection of the cervical glandular surface. It may occur independently of pregnancy or as a result of the traumatism of labor. The common agents of acute and chronic disease are the gonococcus, the streptococcus and the long-continued irritation of trichomonas with its associated symbiotic organisms. The former is, in the vast majority of instances, merely a surface and subsurface infection, producing hyperfunction and cystic degeneration, owing to duct constriction and round-celled infiltration. The secretions are usually purulent, but may later be merely superabundant or even show no appreciable departure from the normal. The postpartum cervical changes incident to an acute infection were described by me* where a streptococcal film is frequently seen over the cervix in the early days following delivery. As chronic endocervicitis is the common sequela of an abnormal puerperium, a few lines devoted to the subject may be enlightening.

It is variously estimated that from 50 to 70 per cent of women show a chronic endocervicitis following pregnancy. I was one of the first to draw the attention of the medical profession to this abnormality. It is not surprising that it is so frequent. The condition of the cervix and its mucosa immediately after labor, especially after first labors, cannot but cause one to respect the great recuperative power of damaged tissues in the puerperal state. When one sees the ecchymotic endocervix, torn from its moorings, frequently hanging like a veil, bloody, bruised, and lacerated, and the fibromuscular tissues torn, divulsed, gray and necrotic by counterpressure from pelvic bony structures against the presenting part, we repeat, one is struck with admiration at the recuperative power of the puerperal woman. A repeated examination five or six weeks later, in a large number of cases, shows the miracle. But the miracle is seldom complete. The fibromuscular structures, like all mesoblastic tissues of the body, have a larger recuperative power than a more highly specialized tissue. Consequently, the glandular tissues suffer more and recover less of their high specialization. Columnar surfaces are the great weakness of the body. It is from these that most diseases find their portal of entry, or local permanency, so that local infections of their surfaces have a faculty of becoming chronic, irritative and vitiative of normal functions.

The diseases of the nasal mucous membrane bear a close resemblance to those of the cervix uteri.

*American Journal of Obstetrics & Gynecology **16**: 339, 1928.

Chronic Endocervicitis.—The picture of chronic endocervical disease from a microscopic standpoint varies greatly with its intensity and duration. In the mildest form there is merely increased activity of the surface columnar epithelium; chiefly that part nearest the cervical canal is most profoundly affected. The deeper one recedes from the canal into the digitations of the glands, the more normal the columnar cells become. This is quite the commonest picture.

The Hypersecretive Types of Endocervicitis.—If we examine the columnar cells more closely near the canal, we find that the goblet part of the cell, that is, its free margin, is more ragged, and is expelling huge quantities of mucus (Fig. 1). The nucleus has moved away somewhat from the base of the cell, and may occasionally ascend as far as the middle, leaving a clear space underneath it. Tinctorially the nucleus does not stain so deeply. A drop of this cervical secretion, pressed between a slide and cover slip, shows a most impressive

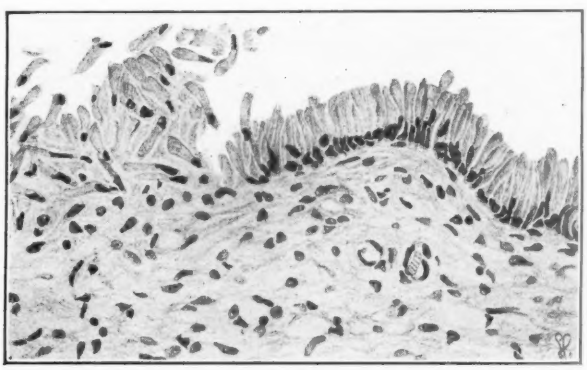

Fig. 1.—Showing to the right marked hyperfunction of the columnar cells, with desquamation of the cells by a sort of explosive excess of function.

picture of thousands of mucous masses shaped either as clubs, sausages, or ovoids, lying in a stream of thinner matrix, with their long axis in the stream line. Each globule has a surface of higher tension which causes it to retain its shape until this is dissolved. These globules are highly granular, and are very beautiful and highly refractile. They may be easily mistaken for the highly refractile encysted types of trichomonas. But their arrangement in streamline fashion at once affords a differentiation. In the more advanced and more intense chronic infections, this state of hyperfunction expressed as hypersecretion may cause a complete explosion of the secreting (Fig. 1) cells, with or without attempts at repair in the deepest basal (Fig. 2) membrane. In still other more acute stages, a wholesale blight may affect all the columnar cells, causing them to be cast off in a state of partial disintegration, not only of the surface, but of all the deeper glands. Secretion is not a prominent feature of this stage, rather destruction captures the attention.

Hyperplastic Type of Catarrhal Endocervicitis.—In other cases, hyperplasia of the glandular linings becomes the predominant feature. This may spend itself chiefly upon the glandular elements affecting the

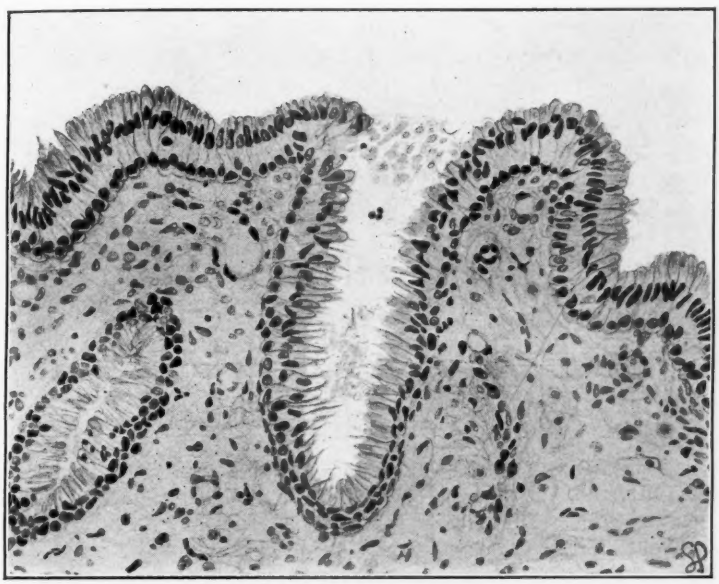

Fig. 2.—Catarrhal endocervicitis. Note the duplication of columnar lining. The more superficial layer is being desquamated by the deeper layer under the stimulation of disease. The underlying tissues are edematous.

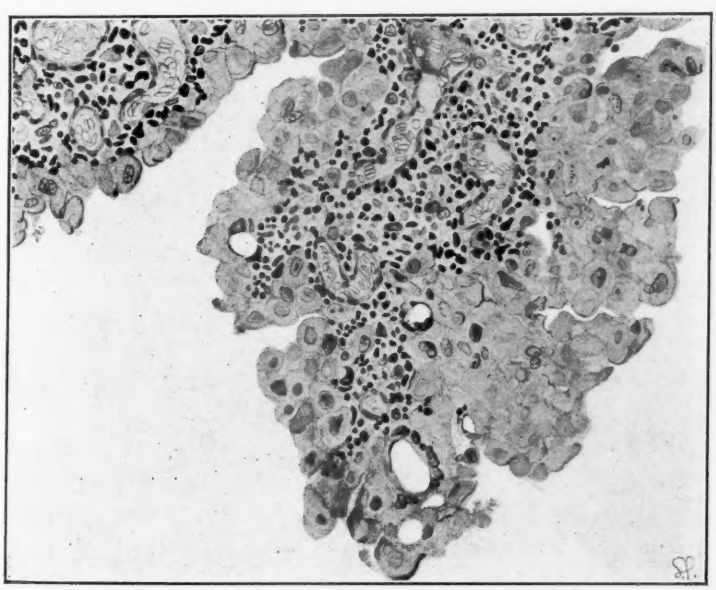

Fig. 3.—Subacute inflammation of the endocervical tissue. There is round-celled infiltration, great vascularity and inordinate multiplication of the columnar cells that are often multinucleated and syncitial in character.

fibrous tissue minimally. Hyperplasia of the glandular elements results in a many layered covering, instead of a single layer (Fig. 3). In many instances, this gives rise to a heaping up of tissue in which secretory function is partially, or totally, lost and cell energy seems to spend itself chiefly in division. These can so closely approach a cancerous stage as to qualify as precancerous, a vague term without justification. The cell division may affect either the columnar cells or the squamous covering. These two types of cells may so revert to their embryonic characters as to be indistinguishable. The columnar cells lose their columnar mucus-secreting characteristics, and the squamous cells cease to be keratinized, become active, and the surface cells are shed before they can become adult as in the normal state. The underlying fibromuscular stroma is usually but slightly affected by the mild infection.

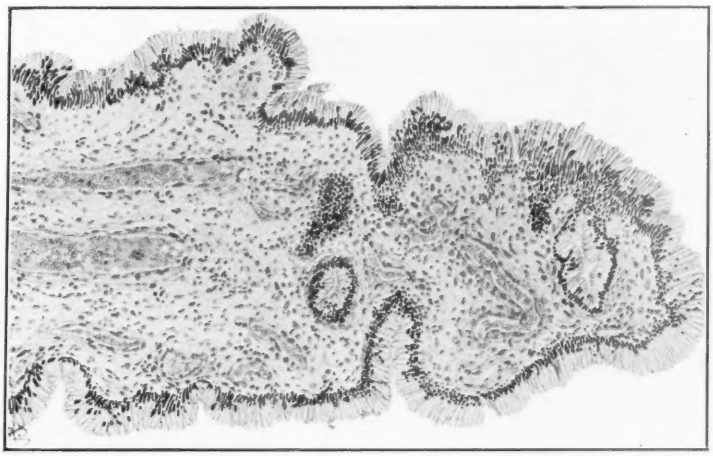

Fig. 4.—A polyp of cervical canal, showing catarrhal changes in the columnar covering cells, edema of the fibrous core and multiplication of blood vessels, a vascular edematous polyp in which both supporting and special cells are involved in a vascular hyperplasia.

Acute Interstitial Cervicitis.—It will be readily understood that the division of cervical diseases into catarrhal and interstitial, and into acute and chronic types, is a purely arbitrary one for descriptive purposes. These types pass insensibly from the one into the other. But the division is one that is generally accepted for similar disease processes in other parts of the body.

Where the inflammatory attack spends itself upon both the lining and the deeper tissues (predominantly postpartum cases) in addition to the mucosal changes, described above, one finds diffuse round-celled infiltration of the deep tissues, with the development of large cervical polyps (Fig. 4), frequently much pedunculated, with an infected, desquamated surface at certain points, and a loose edematous fibrous core. Thrombophlebitic changes are frequent and conse-

quent diapedesis or large venous hemorrhages may occur, not infrequently followed by sloughing. Cystic disease of the glands usually occurs only in the chronic state.

Chronic Interstitial Cervicitis.—This is usually the result of an infection following full-term labor or abortion. The characteristic clini-

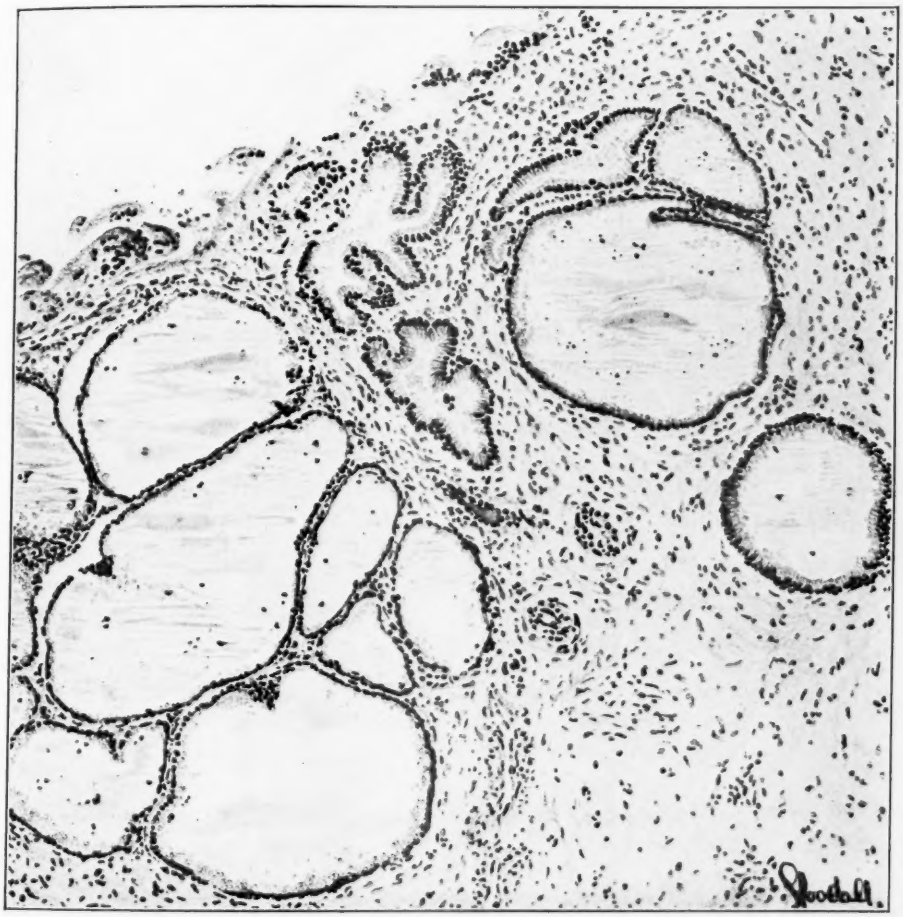

Fig. 5.—Showing eroded surface and multiple cystic disease of the cervical glands, with pressure degeneration of the lining cells. This is at the external os.

cal signs are an inordinately large cervix, the result of chronic subinvolution, with numerous nabothian cysts, which greatly add to its bulk (Fig. 5).

Owing to the hyperplasia of connective tissue and the frequent collections of lymphocytes resembling lymph nodes developed, we think, from the reticular tissue lining the lymph spaces, fibrous constriction of the ducts of the racemose glands occurs, and retention cysts occur

as a consequence of secretory pressure. When the intracystic pressure equals the cellular secretory pressure, then secretion ceases and regressive atrophic changes occur in the lining epithelium, causing all degrees of flattening and frequently complete atrophy of the lining cells. The contents of the cysts may vary from thick tenacious mucus at first, later, purulent, or inspissated white mucus, or liquid clear contents. These different results are the consequence either of the type of infection, or of the duration of the cysts.

There is one feature of this cystic disease that has not been realized. This is the frequency with which the cervical glands at the internal os are affected. Cystic degeneration of the glands at, or near, the external os are visible and therefore frequently described, but cystic disease of the internal os with partial occlusion of the canal and imperfect drainage are very frequent occurrences, in fact, almost as common as the discreet type, but of infinitely more interest because these can arise from both an ascending or a descending infection. The great frequency of old cystic disease of the internal os has another interest. In a descending infection of tubercular origin there are certain points of predilection or sedimentation where the disease metastasizes. Notably one of these is the region of the internal os. Perhaps it is imperfect drainage which determines this. Doubtless there is always delay at any constriction and the internal os is a very decided constriction of the uterine cavity, and, where stasis occurs, circumstances favorable to deranged function and infection must follow. Cystic disease of the internal os, now recognized as a very common condition, has a profound effect upon treatment which will be dealt with at a later period.

Ectropion.—Ectropion, commonly described as erosion, cervical ulcers, et cetera, is a reddened area about the external os, usually concentric with it, varying in size from a small area the size of a five-cent piece to an extension that may involve the whole portio. Although mostly concentric with the external os, it may involve chiefly the anterior lip in an anteverted, or the posterior lip in a retroverted and retroflexed uterus. These differences of spread are accountable to degrees of vascularity. The origin of ectropion is interesting. It can never exist without antecedent endocervicitis from which it springs, and it cannot be cured without first or simultaneously curing the endocervicitis. Endocervicitis is primarily an infection, usually a chronic one ab initio, or degenerating into a chronic state from an acute one. Infection determines blood to the part, which, if prolonged, leads to the development of new capillaries. This congestion leads to certain defects of function, which expresses itself in hyperfunction (leucorrhea) or hyperplasia (division) or both. Hyperplasia does not necessarily have to be accompanied by hyperfunction, so that grave states of endocervicitis may be devoid of signs of leucorrhea. When congestion

has gone on for a considerable period, it leads to hypernutrition or deranged nutrition. The changes described above under chronic endocervicitis now develop. It is only a matter of time before the squamous epithelium, covering the portio, begins to feel the effects of this congestion, and the part that will feel it most forcefully will be the tissue in the immediate neighborhood of the canal, namely, the external os. The basal layer of the squamous epithelium here begins to multiply under the stimulus of hypernutrition. As the division is speeded up, the cells have not time to become adult, imbricated and keratinized before they are pushed off by the press of new cells. The result is a reddened area made up of granulation tissue composed of embryonic squamous cells. This process gradually, but very slowly, extends to give rise to an increasing area of ectropion. If one examines such a section microscopically, beginning from a normal portion of the cervix and thence gradually approaching the region of the internal os, one finds the squamous layer gradually losing its thick keratin layers, and the whole covering growing thinner until at the margin of the ectropion there remain only the basal layers, arranged as deeply staining vertical cells with a layer or two of embryonic cells covering these. Further on, only a single layer remains, and, still further, even this disappears, leaving only occasional islands of cells of squamous origin on a granulating, small-celled infiltrated surface. Islands of highly modified squamous cells may still be found here and there, especially in the neck of the glands which open onto the surface. It is from these that recovery takes place when effective treatment has been applied.

It may be stated with assurance that the common teaching that under inflammatory stress columnar covering may replace squamous epithelium over an area of ectropion is not only wrong theoretically, but also wrong in our experience. Our specimens were taken entirely from the total hysterectomies at operation. The vagina was washed out with a gloved hand only, and liquid green soap. Immediately after the operation the uterus was opened lengthwise, examined, recorded, and a strip about ⅓ cm. thick was then taken from the fundus to the portio, and immersed immediately in bichromate of mercury in formalin. In the majority of cases this strip was cut in one section. If too long for section, it was cut across above the internal os and its continuity was maintained on two slides instead of one. Under these most favorable conditions, where tissue was not more than one hour out of the body before being hardened in its easily penetrated strips, it can be definitely stated that normally squamous cells end abruptly where columnar begin. The transition is from one definite type to the other in a clear-cut line (Fig. 6). In the normal there is no room for any doubt on this point. Second, squamous lining normally dips down into the gullets of those cervical glands

which open onto the squamous portion. This is for protection of the more delicate columnar lining. Third, there is no definite line where columnar lining ceases in the region of the external os. It varies in the height it may invade the cervical canal in different individuals. Fourth, columnar epithelium never replaces squamous epithelium where the underlying soil is hostile to the more hardy

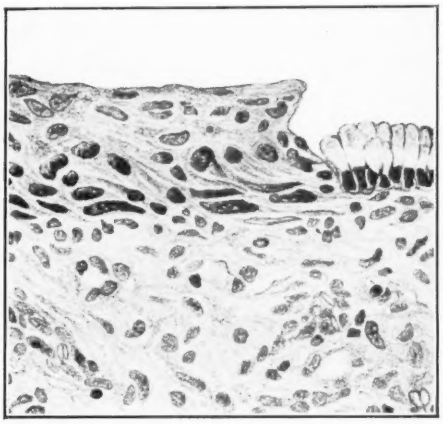

Fig. 6.—Normally the transition from squamous to columnar cells is abrupt.

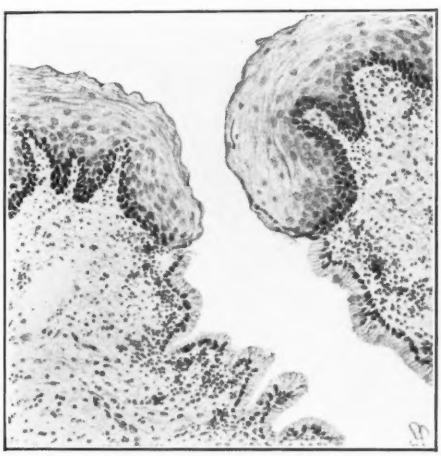

Fig. 7.—Where cervical glands open on the portio, the more hardy squamous lining dips into the mouths of the glands to protect the more delicate, highly specialized columnar lining.

squamous covering. Where squamous cells cannot survive, it is not possible that a more delicate columnar cell could accommodate itself. Under certain circumstances of change, linear arrangement of the basal layer of a squamous covering may simulate that of columnar cells which are undergoing hyperplasia. But this cannot be interpreted, by any stretch of the imagination, into an interpretation of a replacement of squamous by columnar cells. We repeat, such a con-

tention is not only illogical, but contrary to experience. Wherever there is much surface contact with externals, nature interposes squamous epithelium as the safest guard, and the transition from squamous to columnar surfaces is proportionate in degree to the diminution of these contacts. This is a general rule of physiology. Columnar surfaces, and transitional epithelia, on the other hand, under the influence of irritation and frequent contacts, can become hardened and stratified to resemble a squamous covering, but serration and other features are generally absent. The cervical canal and the distribution of its cellular elements will repay a consideration. The canal varies considerably in shape and size under varying circumstances. Normally, it is fusiform, small at top and bottom with a slightly wider center transversely to the body. The anterior and posterior walls lie in contact, each with its frondlike arborvitae arrangements of its folds which by their inclination favor downward drainage. The glandular elements penetrate more deeply into the substratum and are more numerous near the external os. A few open onto the portio. Glandular elements grow less penetrating and with fewer digitations, the nearer one approaches the internal os. The glands are set in adult fibromuscular tissue. One does not encounter the specific interstitial cells of the endometrium until well beyond the internal os, and as soon as one meets these cells, the imbedded glands change immediately into specific uterine tubules.

The depth of tissue involved in glandular invasion at the internal os is singularly thin, and, in abnormal states, is frequently cystic. One can readily see how easily the region of the internal os can be injured and cicatrized by inflammation or traumatism, where glandular recuperative power must be slight where it is so scant.

Under inflammatory disease the canal gradually widens. In nulliparae and virgins this widening involves chiefly the middle portion. The internal and external orifices are more resistant, owing to their greater protection by the uterus above, and by the squamous covering below. The result is a craterlike enlargement of the canal with stasis of secretion or blood. At postmenstrual periods stasis increases the effect of infection. Eventually the periphery of the external os becomes secondarily involved in ectropion and the os widens spontaneously. Presumably similar changes take place at the internal os. It is common experience to find both the external and the internal ora open widely when one cauterizes nulliparas or virgins where prolonged cervical disease has been present. That the cervical disease eventually invades the endometrium by extension upward, we have abundant proof, which will be reserved for another work. That it produces ectropion by extension downward admits of easy demonstration. The surface layers of the endometrium, however, remain immune to this upward extension, owing to their desquamation and re-

newal at each menstrual phase. The upward extension of chronic endocervicitis is limited to the deep layers of the uterine mucosa.

CAUSATIVE AGENTS

There are immediate and contributing agents. The immediate agents are of course microbic. These are chiefly streptococcic, but also gonococcic, trichomonas symbiosis, and rarely of other types. Contributing factors are of the greatest importance. Of highest frequency, of course, are the results of full-term labors and septic abortions. In the former, the traumatic and disruptive influences are the agents which supply the favorable soil. But defective drainage during the recovery stage lends an additional disadvantage. When a woman lies supine, the vagina drains uphill at an angle of about 35 degrees. As a consequence, the traumatized cervical mucosa lies in a cesspool of lochia, from which, after forty-eight hours postpartum, many strains of microorganisms can be cultivated. Each succeeding pregnancy adds its quota to the initial invasion. Carcinoma of the cervix is a rarissima avis in the nullipara. It is a disease restricted to the parous woman. One might almost say, uniformly so. The cause lies not in accidental tears of the cervix, but in the irritation of a chronic endocervicitis or its consequent ectropion. Tears of the cervix play no part in this process, except as a contributory factor in exposing the delicate cervical mucosa to traumatism, or in making it more easily accessible to infection. A great deal too much importance has been attributed by clinicians to cervical tears. Their repair is a matter for profoundest consideration, not only as regards the dangers, but also owing to the almost complete futility of the operations that are commonly used for this purpose. Another contributory cause is found in that type of individual whose mucous membranes are all susceptible to overgrowth, tonsils, adenoids, intestinal and endometrial. By heredity, or acquisition, they are prone to develop disease of the mucous membranes. Other chronic diseases which lower the general resistance may be strong contributing factors in the development of cervical disease.

SYMPTOMS AND SIGNS

The symptoms of endocervicitis are local, extensive and general.

In the nonpuerperal state, acute endocervicitis produces at first leucorrhea, copious and purulent. Later, there frequently develops a feeling of weight in the pelvis. Menstruation may be retarded and then prolonged and profuse. How much of these last symptoms may be due to involvement of the endometrium and appendages, which may develop by extension, it is impossible to tell. Pain, in uncomplicated inflammatory cervical disease, is conspicuously absent. Extension frequently takes place to the endometrium, which is not diagnosable until the appendages are reached. Pain now becomes a conspicuous

symptom. Local or general peritonitis of a mild or severe degree follows. Every acute case tends to become chronic in time. Occasionally spontaneous cure follows immediately upon the acute stage. General symptoms of malaise, backache, loss of appetite, indicative of a toxemic state may or may not follow. Occasionally, though fortunately rarely, general blood infection, without appreciable involvement of the pelvic organs other than the cervix, may develop, causing a general septicemia without localization, or multiple involvement of synovials, endocardial, pleural, meningeal and lymphatic. Pyemia is rare.

In the acute puerperal state there are all degrees of activity. In the majority, the disease remains a local involvement of the cervical mucosa and its lacerations. There are no distinguishable symptoms in the early days of the puerperium. The appearance of the cervix after the third day, described in a previous article and lecture before the Philadelphia Obstetrical Society, shows an edematous state, frequently with a film, a streptococcal membrane over the visible part of the cervical canal. This may be easily wiped off. Symptomatically, however, one finds that the puerperal temperature does not remain normal. There is a daily rise to 99° or 100° F., over many days, with a total absence of pain. This is indistinguishable from a low grade pyelitis, or an inconspicuous pelvic thrombophlebitis. Forty per cent of cases of pyelitis are pain-free. Pelvic thrombophlebitis may be suspected but cannot be diagnosed, except by its mechanical complications. So the mildly febrile pain-free cases in the puerperium may be either acute endocervicitis, pyelitis, or thrombophlebitis. In about 90 per cent of cases the correct diagnosis will be endocervicitis. It is not advisable to expose the cervix and confirm the diagnosis. The cervix is retarded in its involution, as is also the superimposed uterus. This applies equally to the ligamentous structures. Extension of cervical disease may be by continuity of mucous membrane, or by the lymphatic channels. In the former type, pain due to peritoneal involvement is frequently late in development, often involving weeks, with the interval of only comparative well-being. May we outline a recent case the better to illustrate this important, and all too frequent sequela of labor?

I was called in consultation to see a case which had been delivered in hospital one month previously. Her labor and puerperium had been clinically absolutely normal, and symptom-free. She went home on the twelfth day. Ten days later, she had a severe pain in the pelvis, midline, which subsided after one-half hour. Two days later a similar attack developed. This was repeated two days later, but it did not subside this time. Her doctor sent her back to hospital. When I saw her, she had an acute abdomen limited to the lower half, with exquisite tenderness especially over the right side. Naturally, owing to the recent labor, one thought of pelvic complications. Vaginally and rectally nothing but a fullness in the pelvis with great tenderness on imparting movement was elicited. Leucocytes 22,000. There had been nausea and vomiting in the last six hours. Appendicitis could not be ex-

cluded, but pelvic peritonitis was suspected. The abdomen was opened along the right rectus. The appendix was free in its proximal two-thirds, but was involved secondarily between two swollen, indurated tubes, curved each about its ovary. The appendix was liberated and removed and the abdomen closed. The patient made an uninterrupted recovery. There was absolutely no history of gonococcal infection.

It is now becoming more and more recognized that such ascending infections of a mild streptococcal nature are exceedingly common. They are insidious and slow in development. They have been of frequent occurrence in my experience. Smears showed only streptococci. Most of these are mistaken for gonococcal disease. They run a similar, but milder course usually. Sterility may follow, but not so consistently as in gonococcal disease. In the cases where the endocervicitis spreads by lymphatic extension, the first sign of involvement of the peritoneum usually comes on during the early puerperium. A sudden severe state of pain in one or the other lower quadrant, more frequently the left, usually leaves no diagnostic doubt. There is tenderness and a sudden rise of temperature which subsides under appropriate treatment.

Examination, both bimanually and by exposure of the cervix, of all puerperal cases five to eight weeks postpartum, should be a routine. Under these circumstances endocervicitis will reveal itself by a prolonged lochia, followed by a copious leucorrheal discharge, probably a degree of subinvolution of both cervix and uterus, very likely tender uterosacrals and backache. On exposure the cervical mucosa secretes a tenacious clear or semipurulent plug. The canal is more patulous than normal and the mucosa has an unhealthy edematous, pale red appearance. In the chronic state, which is by far the most common type that one sees, the patient usually complains of a leucorrhea of long standing, but growing progressively worse. In young girls and nulliparas, this may be the only symptom. On examination there is a velvety feel about the os which is characteristic of ectropion. When this is present the external os assumes proportions of patulousness that are unusual in nulliparas. When the condition has not reached the stage of ectropion, one frequently finds the external os closed, but, on opening it with a forceps, a great quantity of tenacious mucus from a largely dilated, craterlike canal is obtained.

The ectropion, when present, may be slight or extensive, but rarely exceeds a ten-cent piece in size, in these cases. It is red, granular, and bleeds when cleansed.

In multiparas the process is usually much more extensive. Each pregnancy and labor adds its quota of exacerbation. The leucorrhea becomes more abundant and more troublesome during pregnancy. The patient may complain of backache, fatigue, and poor health. That

poor states of health may be due to this "cervical tonsil" is confirmed by the great improvement which follows upon effective treatment of this condition.

There are two types that are easily distinguished. In the first, the disease is confined almost exclusively to the involved mucous membrane and its ectropion, *chronic catarrhal endocervicitis.* In the second

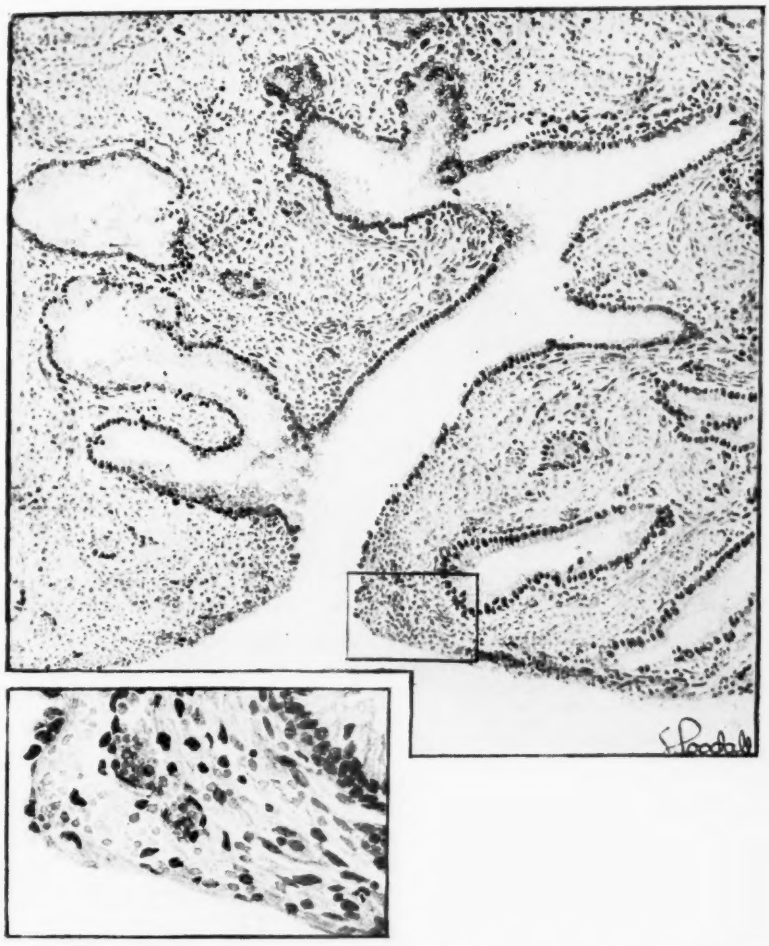

Fig. 8.—*A,* Surface erosion of the edematous, hemorrhagic type without much round-celled or leucocytic infiltration. *B,* The same magnified; note absence of covering columnar epithelium and great vascularity.

type, the cervix as a whole is involved, *chronic interstitial cervicitis.* This distinction is of paramount importance. There are intermediate cases, but generally speaking, they fall conveniently into these two big groups.

Endocervicitis with Ectropion in Nulliparas.—The cervix is more patulous than normal. There is a copious, clear, tenacious discharge;

occasionally this is white with mucous corpuscles, or even semipurulent. The mucous membrane of the canal is occasionally pale and edematous, or red and granular; sessile or pedunculated polyps may fill the visible portion of the os (Fig. 8). The area of ectropion may be extensive, usually concentric; granular and bleeding points are frequent when the surface is cleaned. Frequently a large vessel will emerge from the granular ectropion and course outwardly to the periphery. These sometimes assume large proportions. At other times the outer margin of the ectropic area presents one of two characteristic pictures. In the one, there is a blueness at the periphery as if there were venous congestion below the thin squamous margin. In the other, there is a white rim of heaped-up epithelium surrounding the ectropion as if sugar-coating had been applied. These differences arise out of differences of congestion and lack of balanced desquamation.

When the cervical canal is invaded for treatment, its irregularities, owing to enlargement and sessile overgrowth, become apparent, and dilatation of the internal os is frequently so wide as to give the impression of passing insensibly into the uterine cavity without definable limit to the canal.

These findings of irregularity in the cervical canal, owing to overgrowth of epithelium, may vary greatly in degree. In many cases the endocervical infection will spend itself chiefly in hyperfunction expressed in an abundant discharge. The difference is one inherent in infection and local resistance.

Chronic Cervicitis.—This is a disease of the parous woman. It is a sequence of an abnormal puerperium, whether full term or postabortum. The distinguishing features of this disease lie in the large dimensions of the cervix, its hardness and its associated cystic involvement. It is a disease of multiparas, in which, owing to involvement of all the cervical tissues in infection, or owing to some general debilitating cause or causes, the normal involution of the cervix has been incomplete. The dimensions vary considerably from a slight enlargement to proportions that fill the vault. Hardness is a characteristic. Nabothian cysts are the rule and frequently stand out as sagolike bodies over the portio. The cervical canal is tortuous, granular, and hard. Everything seems fibrous. The uterine body is similarly involved in a state of chronic subinvolution, as evidenced by a large globular symmetrical, usually tender corpus. This condition is frequently described in textbooks as "chronic metritis," "chronic fibrosis uteri" and "arteriosclerotic uterus." Endocervicitis and ectropion usually accompany the cervicitis but these may not be prominent.

It is most important to distinguish the cases that are dominantly endocervical from those that are dominantly cervical, because the treatment is essentially different.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of inflammatory diseases of the cervix from other conditions lies essentially between advanced cases of chronic inflammatory disease and incipient carcinoma. We know of no rule, except clinical experience and biopsy.

The insidiousness of the change from inflammatory disease to new-growth cannot be better illustrated than in the records of St. Mary's, where, in the pursuit of this work, two cases of unsuspected carcinoma of the cervix were found in the microscopic study of uteri removed by total hysterectomy by the authors, in the past year, roughly, somewhat over 2 per cent was found in 90 cases. Two others were placed in the category of precancerous hyperplasia, a rather loose term to designate overgrowth with solid masses filling the acini, but no evidences of breaking through the normal external glandular boundaries.

TREATMENT

The whole of this treatise has for its object a plan to outline a *logical* treatment of inflammatory cervical diseases. The conclusions that are about to be expressed are based upon a studious knowledge of pathology and a wide clinical experience. It is an expression of very firm convictions.

The subject may be dealt with most convincingly under three headings.

1. Nonsurgical treatment.
2. Surgical treatment.
3. The influence of a residual inflammatory cervical disease upon pelvic operations.

1. *Nonsurgical Treatment.*—There are very few forms of treatment of the inflamed cervix that offer any hope of recovery or even amelioration other than the thermocautery. Prior to its introduction, the medical profession leaned strongly to preparations of tincture of iodine, carbolic acid and other escarotics. Later, diathermia and "Elliot" enjoyed a vogue. These have been abandoned for the more rapid, more controllable and more effective electric thermocautery.

It is chiefly to the efficacy and limitations of this presently universal treatment that we wish to draw particular attention. Thermocautery is a very effective form of treatment when properly applied in suitable cases, especially if one does not wish to destroy cervical function. Where, on the other hand, one wishes to destroy function, the matter is quite another affair. Under these circumstances, cautery must be deep and thorough, or, if the cautery knife be used, it must go deep enough to destroy all the glandular tissue. Thoroughness is here the keynote.

However, in those more numerous cases where cautery is used to re-establish normal cervical function, the limits of this form of treatment

are very circumscribed. An excellent result can be anticipated only in those cases that are mild, and limited chiefly to the surface epithelium lining the cervical canal: chronic catarrhal endocervicitis. It is most effective in nulliparas, and in early treatment after each delivery. It becomes progressively less effective the more widespread the disease, the deeper it has involved the cervical stroma, and the greater the organic departure of the cervix, as a whole, from its normal size and consistence.

It has been stated, in the subject of pathology, that in the great majority of the milder cases the pathologic changes are more pronounced at the canal surface, and that the glands are more normal the deeper one recedes into the cervical parenchyma. This can be demonstrated in a very large percentage of chronic mild or recent subacute inflammatory implantations. This is just the opposite of

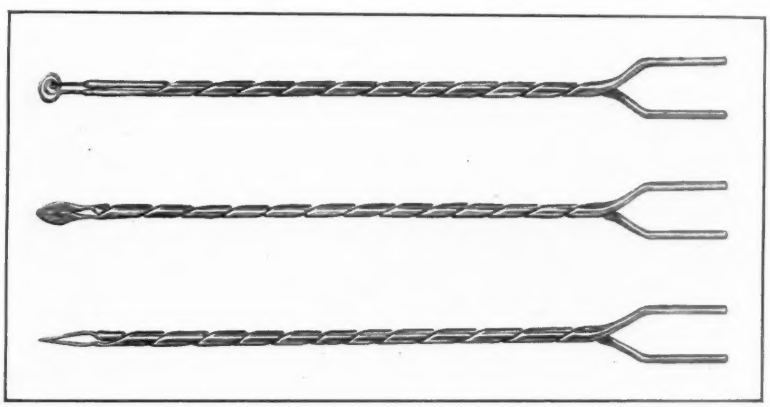

Fig. 9.—Cautery points used by authors in the cure of chronic catarrhal endocervical lesions. The instruments are heated only at the extreme one-half inch. The shank is never more than warm. Natural size.

what happens in the endometrium, where the surface sheds its diseases but allows them to become permanent in the deeper layers. The cervix, therefore, conforms to the general rule of tissues. The uterus is the exception, owing to its special function.

Now, it is just in these mild chronic cervical canal diseases in nulliparas and in recent subacute infections that treatment has proved most effective. In such cases logical treatment destroys the surface layers, and allows the deeper-set normal glands to regenerate a new lining, which they will do, most effectively, if not destroyed. A minimum of scar tissue is caused by such treatment. In nulliparas, one should be careful not to cauterize the external os overvigorously where there is no ectropion. Where, on the other hand, there is ectropion, there is no such risk because the larger the area of ectropion, the more will the external os be patulous and soft. In cauterizing the canal it is not necessary to cleanse the canal of mucus

with caroid or other solvent. In fact, fluid in the canal is a great advantage, for, when it boils, it distributes the heat equally and generally over all the interstices of the canal, so that there is no necessity of bringing the cautery point into contact with the canal epithelium at all. When the canal presents a whitish or parboiled appearance, the treatment has progressed far enough. Treatment so effected will not produce a pocketed and tortuous cervical canal, because the heat is evenly distributed. Frequently, in the next ten days, a thin cast of the canal is shed or the necrotic tissue is disintegrated, leaving a pink healthy mucosa. Care not to constrict the external os is effected by using the cautery type as illustrated below (Fig. 9), where only the wire top is heated. In these cases especially, but preferably in all cases, the cautery should be introduced cold, having previously ascertained the degree of current necessary to effect a dull red heat. The objects of the cauterization are not only to destroy the diseased cervical mucosa, which is the seat of irritative hyperfunction or hyperplasia, but also to destroy the subjacent hyperanemia and hypervascularity, which can be done effectively only by a dull penetrating heat. A white heat will not only just scar the surface, but will destroy the platinum points. With proper care these delicate nasal cautery points will last for years. One minute of careless white heat melts the platinum, and destroys the whole instrument. If ectropion has developed, this should be treated by radical tracings from the os outward to, and beyond, the margin of the ectropion. This again should be done in a dull red heat, by a fine knifelike platinum point. The knife should move slowly over the surface and should penetrate only about $\frac{1}{16}$ of an inch, but slowly enough to coagulate the supernumerary subjacent blood vessels. When larger, discrete vessels emerge from, and course over, the area toward the periphery, these should be destroyed with a finer cautery point, at their point of egress. Similarly nabothian cysts should be punctured and the contents caused to boil with prolonged contact. Sterile paraffin is then applied to the cervix and vagina. Douches are not begun until twenty-four hours after cautery. Their efficacy is questionable, but they are generally used. The pressure should be minimal, and lactic acid, 2 drachms to the quart, is very effective.

Cauterization of the cervix should be done as soon as convenient after cessation of a menstrual period. By choosing this time there will be less bleeding, better healing, and less disturbance of the succeeding menstrual phases. If treatment is instituted after the middle of the intermenstrual period, the subsequent menstruation may be greatly advanced, together with an increase in duration and quantity. In cases where there is much edema of the cervical lips and an unhealthy pale appearance of the area of ectropion from uterine allergy, cardiac disease, or other cause, the efficacy of cautery may be greatly reduced.

Complete restoration of normal function should be effected, together with pearly whiteness of the ectropic area, in about thirty days. Any disease of the appendages, chronic or otherwise, is a contraindication to cautery. Vaginal and vulvar infections should receive appropriate treatment before applying cautery to the cervix.

Cautery of the cervix is contraindicated in gonorrheal disease, except where it is confined to the cervix, a rare condition and a very difficult one to determine. However, I have seen two cases of gonorrheal septicemia, and one of gonorrheal inflammation of the interosseous membrane of the forearm, cured by cautery of the cervix, where there had never been any peritoneal symptoms or palpable disease of the adnexa. Acute and subacute cervical infections may act as a tonsil, constantly infecting the pelvis with successive bouts of peritonitis and involvement of the appendages. Articular disease may not infrequently owe its origin to an infected cervix. There is a certain risk in cauterizing such cases, but it may be undertaken with caution when the metastatic state is grave, and seems to warrant it, and where the cause and effect have been established beyond doubt. Pelvic abscess after cautery, where an unrecognized appendage disease or a virulent infection is present in the vagina, is not common, but always a possibility. The writer once saw a virulent streptococcic septicemia with a rashlike scarlet fever follow upon a mild cauterization.

2. *Chronic Cervicitis.*—We wish to emphasize not only the inadequacy, but also the harmful results, that may follow deep cautery of the cervical canal with a large cautery or cautery knife, extending from ¼ to ⅓ inch in depth, in the hope of reducing a large hard cystic cervix. In the first place, pathology shows the futility of this procedure, and in the second place, it so cicatrizes the cervix that dilatation at subsequent labors may be greatly inhibited or impossible. Cautery of this heroic type tends to produce cervical cicatricial transverse bands with irregular pockets of secretion, tending to aggravate the progress of the original disease. Cicatricial disease of the internal os, where the mucosa is normally very thin, may lead to hematometra and pyometra. There is no cure for such a large fibrous cervix. Its bulk will greatly reduce after menopause, natural or artificial. Practically all types of cervical inflammatory disease rapidly improve after the menopause, with the cessation of the congestion and flow of menstruation.

3. *Surgical Treatment.*—Surgical treatment for the cure of inflammatory disease is an ostrichlike subterfuge. It removes the disease from the scanning eye, but it bottles it up in the canal. It is almost impossible to remove all the diseased mucosa by the deepest coning out during amputation. Drainage is frequently blocked and healing is frequently only by secondary intention. Amputation of the cervix,

for the cure of endocervical disease, is as dead as the dodo in the larger clinics, but is still all too frequent in the smaller centers and hospitals.

But the worst features are the two complications which so frequently follow upon the cervical operations in the presence of endocervicitis. These are (1) late septic hemorrhage and (2) lack of primary union. How frequently one sees women enter the hospital in comparatively good health for a very "simple" operation upon the cervix! On the eighth to the twelfth day, and sometimes later, septic, uncontrollable hemorrhage follows which resists packing and secondary suturing, and yields only to two or more transfusions. Before its arrest the patient is reduced to a very critical state and anxiety is written plainly on the surgeon. And following upon this or without its antecedent, septic hemorrhage, the whole wound separates and heals by granulation, frequently complicated by a thrombophlebitis. For years I have lectured against cervical amputations and cervical repairs in the presence of chronic mucosal disease. If a repair is deemed necessary, owing to tears (undue importance is attached to these) and there is an associated endocervicitis, this latter ought to be cured first by appropriate methods, and, if cure by cautery is impossible, owing to some cause described in the foregoing paragraphs, then repair should be superseded by something less dangerous and more efficacious.

THE INFLUENCE OF A RESIDUAL INFLAMMATORY CERVICAL DISEASE UPON THE RESULTS OF OTHER PELVIC OPERATIONS

The residual cervix in these cases may act in the same manner as would a subacute or chronic gonorrheal case, only with a stronger tendency to produce postoperative septic hemorrhage and thrombophlebitic and lymphatic invasions. We are just beginning to displace the teaching that gonorrhea is a disease sui generis. It has much in common with the more common and usually more penetrating lesions of the streptococcus, especially when the latter has reduced its virulence to the level of the average gonococcal strain.

To show the trend of thought in the past few years, one has but to call to mind the general adverse reaction to leaving a uterus or cervix when dealing with gonorrheal disease of the genital organs. True, operations are infrequent in such conditions. But even today such operations have to be undertaken occasionally, for pain and economic reasons. Experience has taught us that a clean sweep is to be advocated, that is, the removal of all columnar surfaces. Why? Because the residual mucosa may be a constant infected tonsil to the pelvic system. We know today, that many low-grade ascending infections, indistinguishable from gonorrheal disease, are of streptococcic origin, whether they are postpartum or not. Thrombophlebitis is a much more

common complication of pelvic operations when the cervix remains than when it has been wholly removed, because, in the vast majority of instances, thrombophlebitis is a disease of a subacute nature arising out of a chronic or subacute mucosal disease. A little study of results will confirm this. This angle of the problem is fully dealt with in the authors' recent work *Total Versus Subtotal Hysterectomy.** Chronic inflammatory disease of the cervix usually improves after removal of both ovaries, but in conservative operations upon these appendages, followed by a subtotal hysterectomy, the diseased cervix frequently postoperatively develops a vitiated function, to the great disturbance and annoyance of the patient. In the past three months I have had to remove the cervix in three cases after supravaginal hysterectomy, where an anterior or sequent disease of the cervix set up unpleasant intolerable symptoms. These are partly due to the nutritional changes initiated chiefly by subtotal hysterectomies. Destruction of the cervix at the first operation, or its removal by a more radical procedure, would have obviated the sequelae. It is most removed from our wishes to advocate difficult operations with which the operator is not familiar or in which there resides a large element of conservative fear. But other less dangerous means should be adopted to eliminate the cervical postoperative hazard. What procedure should be advocated? Always the one that is best suited to the combined welfare of both patient and surgeon. This must always remain an individual personal question. But let us not close our eyes and ignore the hazard. It must be met, squarely and fairly in the interests of both patient and doctor. The truth is summed up by the confidential statement of a surgeon to me: "It is *very* comforting to both patient and surgeon when the cervix has been rationally dealt with and *most* comforting when that menace has been successfully removed."

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OPERATIVE TECHNIC OF VESICOVAGINAL FISTULAS

JOSEPH HALBAN, M.D., VIENNA

IN THE course of years I have had occasion to perform about eighty operations for vesicovaginal fistula, exclusive of sixty cases operated in the Wieden Hospital to be published shortly by Mestitz. This represents a considerable number, if we take into consideration the decrease in the number of fistulas due to improved obstetrics and the perfection of operative gynecologic technic. A series of one hundred and more fistula operations, such as have been reported by previous authors (Simon, Salzer and even larger series by Emmet, 400 cases, Fritsch, Küstner, Bozeman, each 200), are no longer within the individual experience of any single operator.

My earliest experiences in this field were in the period of my assistantship at Schauta's clinic. The methods then employed were quite different from those of today. The patients were fastened in the knee-chest position to the Bozeman's fistula table. We used bronze wire suture material inserted and tied with a special instrument. The technic has been considerably simplified. We now operate in the ordinary lithotomy position and use fine catgut. To a certain extent each operator must develop individually and each operation enriches his experience. Today, I approach a fistula operation with great optimism and with the expectation of obtaining a primary cure in the overwhelming majority of cases, providing certain experiences are evaluated and certain definite principles are adhered to.

It is a great pleasure and satisfaction to a surgeon to cure a fistula, especially at the first attempt. Failure means torture both for the patient and for the operator. One can appreciate what a blessing the present-day methods have brought about in view of such martyrdom as occurred in the beginning of fistula operations (Defenbach operated 18 times; Wutzer 30 times). The good results are due to the simplification of the technic and adherence to three basic principles:

1. Extensive exposure of the fistulous area.
2. Mobilization of the base of the bladder.
3. Correct suture.

1. The exposure of the field of the fistula is accomplished by the correct application of posterior and lateral retractors. If the vagina is narrow or the perineum is high and rigid, or if the fistula lies deep in the vaginal funnel as so frequently occurs in those fistulas that result from gynecologic operations, such as hysterectomy, the following procedure is absolutely indicated. An initial episiotomy or a unilateral

or even bilateral Schuchardt incision is made which facilitates the approach tremendously. The principle of thus widening the vulva was already emphasized by Jobert de Lamballe; but the deep vaginoperineal incision according to the technic of Schuchardt is as a rule far preferable, offering an additional advantage in the cases of adherent fistulas, as we shall see below.

2. The base of the bladder can be adequately exposed only if it is sufficiently movable. As a result of the original causative traumatic factor (forceps, etc.), we find not only tears of the bladder wall but also injuries extending deep into the parametrial and paravaginal tissue, causing dense scar formation so that the entire base of the bladder can be adherent inferiorly and laterally. Therefore it is absolutely necessary, and this was known to the earliest operators, to liberate the scars immobilizing the base of the bladder, and thus restore its mobility. Stretching of the scars by means of Bozeman's hard rubber plugs is only of historic interest. It is so much simpler, surer, and quicker to separate them bluntly with the finger or with a cutting instrument. If necessary this can be done through a suprapubic incision (Bardenheuer).

Still better and simpler is the procedure of Schauta who recommended a vertical incision external to the labium majus through which he proceeded to free adhesions by blunt dissection paravaginally up to the os pubis, or if they were fixed to the periosteum of the os pubis, he freed them with a raspatory. If the adherent scars are less extensive and in the vicinity of the fistula rather than in the paravaginal tissue, such an accessory incision will not be necessary, and they may be separated in the course of the exposure of the base of the bladder. Formerly I used the paralabial incision of Schauta considerably, with good results. Latterly, as a result of my experiences with vaginal operations for carcinoma, I have arrived at what I consider a simpler and better method, which I have described in my book on *Gynecological Operations*, page 398. On the scarred side or even both sides, I make a Schuchardt incision and then dissect upward with the finger along the mesial wall of the levator ani, which permits an easy separation of the entire scarred area from the levator ani and from the symphysis mesially. If the scarry strands are too dense, a raspatory or a scissors can be used. Excessive bleeding is exceptional; if it occurs, temporary packing with Stryphnon gauze is usually sufficient as the bleeding is almost always parenchymatous and there are no large vessels in this vicinity. The entire lateral wall of the bladder is thus rapidly and easily mobilized and facilitates the mobilization of the floor of the bladder, so essential for bladder suture. The Schuchardt incision has thus an invaluable double advantage in severe cases. It makes the fistulous field accessible and the extensive separation of paravaginal

scars laterally possible, thus facilitating the lateral mobilization of the bladder. If the vagina is particularly ample and the fistula easily exposed, the Schuchardt incision may be avoidable. It is then sufficient to make an incision in the lateral vaginal wall where the levator ani approaches the vagina, and from this point to proceed along the inner surface of the levator to the scars and then separate them. For difficult cases I can only repeat my most earnest recommendation of the Schuchardt incision and the separation of the lateral wall of the bladder from the levator ani.

Mobilization of the base of the bladder itself is occasionally difficult, particularly in those fistulas resulting from hysterectomy. In these cases the fistulas are high up, usually just underneath the scar which closes off the vagina cranially, that is, in the vicinity of the scarry funnel. Such fistulas are adherent to this scar, and it is obvious that they can be sufficiently exposed only by a free dissection of the portion of the floor of the bladder behind them. In this not uncommon variety of fistulas, the scarred vault must be split transversely, separating the anterior from the posterior vaginal wall. Great care must of course be exercised to injure neither the bladder nor the rectum. If the separation is successful, a finger is carefully inserted into the sac-of-Douglas and separates the posterior surface of the bladder from the anterior surface of the rectum. It does not matter if the peritoneum is opened; there is almost never an infection. One can then draw down that portion of the bladder base which lies behind the fistula, in effect what Jobert de Lamballe called "glissement" or "locomotion" of the bladder, exposing it for the correct placing of the sutures. It is obvious that the portion of the bladder base anterior to the fistula must be exposed as well, a much easier task. Small movable fistulas in the middle of the vagina can be healed by the old method of freshening of the margins. I have practically abandoned this procedure, and I expose the fistulas extensively according to the principle of flap-splitting (dedoublement of the French). This is in most cases easily possible if we stick to the principle which I have laid down for the dissection of the vesicovaginal fascia, to wit: that the fascia is permitted to remain on the bladder and separated with the latter from the vagina. One thus remains in the correct anatomic plane, mobilizing the bladder safely and easily. If the fistula is high in the vaginal vault, the mobilization of that part of the base of the bladder which lies behind the fistula is accomplished as I have described above.

It remains a basic principle to expose and mobilize *both* the anterior *and* posterior portions of the base of the bladder.

It is immaterial whether one utilizes a cuff of vaginal mucosa or not (Füth method). Those authors who believe that the turned-in vaginal cuff contributes to the closure of the bladder are certainly in error.

On the other hand, it is not necessary to be concerned about the edges of the fistulas and to try to freshen them. The Füth method is applicable only for the simplest cases. If the fistulous opening is very large or considerably adherent, the construction of a vaginal cuff does not come into consideration. In these cases we must attempt the most extensive exposure of the floor of the bladder.

3. I believe that there is only one correct way to place the bladder sutures, with the best chance for primary union. That is, to ignore the fistula itself entirely in the placing of the sutures and to sew the anterior and posterior portions of the floor of the bladder right over the fistula. These two portions with their attached vesicovaginal fascia are united by fine catgut sutures in areas uninjured by scars or dissection, usually about 1 cm. from the margin of the fistula. One may bury this with a second row, useful but not necessary. The vaginal mucosa may, if desired, be sutured over this. This is quite immaterial as far as the result is concerned. Occasionally it may be easier to place the sutures sagittally rather than transversely. The tension is, however, usually less in the latter and it is thus preferable. The essential point is to sew healthy bladder wall to healthy bladder wall, taking about half the thickness in the suture and not too close to the fistula. I think this is the basis of the good results obtained with Füth's operation. The preservation of a vaginal cuff itself plays no rôle but it necessitates sewing the bladder farther out, and this works out well.

Ever since I have convinced myself, as a result of personal experiences and occasional failures, that the principles in the treatment of fistula operations that I have mentioned above are basic, I have had excellent results and have undertaken the operation of even very difficult and unfavorable cases with the greatest optimism. The principles are not new and are recognized by most authors. They can, however, be followed more easily by observing technical details that I have pointed out above. One can thus get primary results better than our previous ones.

Füth reported 68.2 per cent cures (Kleefisch), Franz over 78 per cent, Schulte over 71 per cent. By following the foregoing rules these results can doubtless be surpassed.

Undoubtedly, exceptional cases occur requiring exceptional methods, as for instance, flap plastics, or the utilization of the uterine fundus, or the cervix for covering, etc. The more one adheres to the principles described, the less frequently will unusual procedures be necessary; even severe and unfavorable cases can be cured by simple suture of the bladder. I have not included in these observations those vesicovaginal fistulas with defects of the urethra or of the bladder sphincter. Such cases require quite special measures for cure, not within the scope of this communication.

CONTRIBUTION TO THE PROBLEM OF HEREDITY OF ENDOCRINE DISORDERS

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IT HAS not escaped the attention of previous authors that hereditary factors were of great significance in the etiology of diseases of the endocrine glands. There are numerous observations and indications in the literature of this fact. It is not our purpose at this time to stress this phase, and we refer the reader to the comprehensive exposition of this subject by Berta Aschner[1] working in our division. In this report it was definitely established statistically that in families in which Basedow's disease occurred, simple goiters were found more frequently than the normal expectancy and vice versa in families in which simple goiters occurred; hyperthyroidism was more frequent than the average, i.e., that there was a genotypic relationship between the two illnesses.

It is well known of most of the endocrine illnesses, that they can appear frequently in one family, be genotypically determined. Among these are myxedema, Addison's disease, hyperfunction of the adrenal cortex with intersexuality, precocious puberty, acromegaly, pituitary dwarfism, etc. The marked hereditary factor in obesity, diabetes mellitus and in rare cases of diabetes insipidus is also well known. The modern science of heredity no longer considers the investigation of the special type of hereditary transmission the primary problem, but instead, lays its emphasis on the detailed study of the smallest inheritable factors, the so-called genes, together with the establishment of other phenotypic manifestations. Disease, of course, cannot be inherited, only a particular anlage which can result in various phenotypic manifestations as a result of diverse endogenous and exogenous influences. The investigation of these various conditions and the discovery as to what really constitutes this inheritable anlage seems to me at present to be the chief problem in the study of human heredity. Perhaps some examples will illustrate the point:

CASE 1.—Gina. No. 2618. A thirty-six-year-old woman with typical Basedow's disease, diffuse struma with thrill. Pulse 132. Basal metabolism rate plus 75 per cent to plus 99 per cent. Mother also had Basedow's disease. The child of this patient died two days after birth and had a large congenital struma.

This case illustrates beautifully the statement by Aschner cited above in reference to the inheritable disease anlage and its disposition to the formation of goiters with or without hyperfunction.

CASE 2.—M. S. No. 6270. A child of six years with typical infantile myxedema which had been recognized for some time. The child was being given thyroid extract constantly with the expected therapeutic result. The paternal grandmother had died of carcinoma of the thyroid.

This case seems to show that a peculiarity, one might say a biologic inferiority, of thyroid is transmitted in this family, in one case leading to early infantile deficiency and in another, to malignant disease.[2]

CASE 3.—No. 5893. This history deals with two brothers of whom one had acromegaly with enlargement of the sella turcica and the other a malignant tumor of the pituitary which had resulted in bitemporal hemianopsia, optic atrophy and obesity and for which he had been operated upon. The daughter of the acromegalic was excessively obese. The nineteen-year-old son of the other was a eunuchoid, extremely tall and fat, with the characteristic hair distribution.

Again we are confronted with the diverse phenotypic end-results of a pathologic hereditary predisposition, influencing the hypophysis in the direction of various diseases. This is another example of the biologic inferiority of the organ in question.

CASE 4.—M. C. Hospital admission No. 7395. A woman of thirty-five years with severe diabetes mellitus. The mother died at fifty-six years of age of Addison's disease, and the maternal grandmother had had diabetes.

CASE 5.—E. von G. Hospital admission No. 5884. A woman of thirty-six years with all the symptoms of Addison's disease, probably not of tuberculous origin. The father had diabetes and years ago had had an operation for tumor of the testicle.

The above observations point toward the fact that illnesses of various endocrine glands may alternate in one family in a remarkable way. Only sufficient analogous material would permit our drawing definitive conclusions. Nevertheless, such observations remind us to pay more attention to this type of material in the future.

CASE 6.—T. K. Hospital admission No. 7157. A girl of twenty was admitted with very extensive hypertrichosis of the face, the breast, the abdomen, back and thighs. Considerable obesity and struma were present. There were no other criteria for the diagnosis of a tumor of the adrenal. Menstruation was perfectly normal. Two maternal aunts had been operated upon for Basedow's disease. Two cousins of the mother suffered from extreme obesity.

This case is closely allied to Cases 4 and 5 and requires no further discussion.

CASE 7.—V. W. Hospital admission No. 6703. A woman of thirty-one years complained of a severe alopecia. In addition, she had quite a mustache and a hypertrichosis of the legs, also a struma, a hypoplastic uterus and she was sterile. The mother of this patient had had similar alopecia for twenty-five years and had had an operation for struma.

It is obvious that this rare anomaly of hair distribution has nothing to do with the goiter. This anomaly of the definitive peripheral end organ is one of the signs of what I have called "endocrine stigmatization" of a constitutional variant, having a general influence on the entire hormonal regulatory system. Sometimes this strikes the gland of internal secretion itself, sometimes its peripheral sphere of activity. The hereditary relationships of cryptorchidism are of particular interest in this connection. This dystopia of the testicle, a retarda-

tion in development, is occasionally found in a number of members of the same family. In addition, it shows quite unmistakable connections with other genotypically conditioned, that is, constitutional disturbances in such families. It is not a mere coincidence to find various disturbances of sexual differentiation in the members of the families of cases of cryptorchidism. These may be eunuchoids in whom the gonads persist in an infantile state, or retardation of development of the gonads secondary to pituitary influence or anomalies of the psychosexual constitution, for instance, various forms of perversion. It would seem, then, that the gene responsible for the development and ripening of the testicle must, in some way, be integrated in another complex of genes which have different functions to fulfill, nevertheless representing a common biologic unity. On the one hand, we are dealing with those *anlagen* responsible for normal sexual differentiation. On the other hand, there are obvious relationships between cryptorchidism and constitutional obesity.[2] As I have repeatedly remarked in more detail,[3] the assumption seems indisputable that the anlage of obesity is to be understood in this sense, that an individual left to his automatic regulation acquires a more or less excessive increase in weight in the form of an excessive fat deposit which fact is inevitably predetermined in the germ-plasm. This genotypic predetermination to obesity shows its end-results on various organs concerned with fat production and the regulation of its deposit. Among these are the fat-storing mesenchymal tissues, a series of glands of internal secretion regulating such storage and certain portions of the vegetative nervous system. The gonads belong to the circle of organs affected by the pathologic disposition to obesity. It is thus, and thus only, that we can understand why retardations of development and other constitutional disturbances of the gonads are not infrequently found associated with constitutional obesity, without the necessity of attributing the obesity directly to the disturbances of the gonads. It is much more likely that it is the expression of an outranking genopathy and belongs to the category of those constitutional variants which we have designated as endocrine stigmatization. The following family histories illustrate this point:

CASE 8.—A. H. Hospital admission No. 5792. Thirteen-year-old boy with abdominal dystopia of the right testicle. Father was a transvestist and sadist, wears lingerie, etc., and a corset. Both the father and grandfather are heavy drinkers.

CASE 9.—E. F. Hospital admission No. 5197. Ten-year-old boy with right cryptorchidism and obesity. Within the next two years, there was spontaneous descent of the testicle. The father of this boy had a very late development and noticed pubic hair only at the age of eighteen. His genitals were always considered ridiculously small. The mother was a short, fat woman, weighing 90 kg. The brother was fat and weighed 5 kg. at birth.

CASE 10.—G. R. Hospital admission No. 7293. Forty-one-year-old woman with marked constitutional obesity, weighed 124 kg. Both parents and a sister were obese. The son was also extremely fat and had a cryptorchidism.

CASE 11.—W. G. Hospital admission No. 4750. Seven-year-old boy, left-sided cryptorchidism. This boy was not obese. The father has marked obesity, though not tall, weighed 109 kg. Numerous instances of obesity in the father's family. Mother weighed 80 kg.

CASE 12.—R. S. Hospital admission No. 5277. Ten-year-old boy, right-sided cryptorchidism, obesity; fat distribution typical of a eunuchoid of this age. The father, paternal uncle, and six of eight paternal aunts were markedly obese. One of these had a son with a typical pituitary dwarfism, without obesity. A cousin had had an operation for cryptorchidism at the age of ten.

The preceding case histories are only a fraction of the material that we have observed in recent years. Because of the relatively small number, they prove nothing and can lead to no definitive conclusions. The sole purpose of these notes is to focus the attention of authors to the highly interesting relationships between heredity and the endocrine glands and to stimulate them to collect sufficient material, so that at some later time an impeccable statistical evaluation will be possible. However, I feel that one conclusion is justified: that it is impossible to ignore the facts gleaned from the study of heredity or to practice endocrinology without a detailed knowledge of heredity.[4]

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MARIANNENGASSE 15.

A COMBINED OPERATION FOR COMPLETE HYSTERECTOMY

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THE respective merits of abdominal supravaginal hysterectomy and complete hysterectomy have been the source of considerable discussion for many years. It seems agreed that there is a slightly greater risk to the patient when a complete operation is performed, though Goodall has recently published a very excellent report, with no operative mortality. In addition to the question of greater operative mortality and morbidity, other problems involved in the decision to perform either a complete or a supravaginal hysterectomy are of importance. The possibility of overlooking a beginning cervical carcinoma while slight is still a danger, and the subsequent development of a carcinoma in the retained cervix, especially when it is lacerated or eroded while infrequent, does occur. Furthermore, a cervix, the site of a persistent inflammation, should be removed. The "coning out" of the cervical mucosa alone is not as complete a procedure as the actual removal of the diseased organ. In other words it is of advantage in many cases to do a complete rather than a supravaginal operation.

Richardson has clearly pointed out the indication for both vaginal and abdominal complete hysterectomy. He stressed that in properly selected cases a skillfully executed total hysterectomy by the vaginal route is a decidedly less formidable procedure than is the same operation performed with equal finesse by the abdominal route. He also states that "in the nulliparas, complicated pelvic pathology tremendously increases the technical difficulties." In addition even in women with roomy vaginas, extensive and dense abdominal adhesions render the vaginal route unsuited for hysterectomy. In the presence of large ovarian neoplasms of solid or cystic type, the vaginal method is also inadvisable. Fibroid tumors when larger than a four months' gravidity, while they may be removed by morcellation, offer considerable technical difficulty. The vaginal approach also precludes the proper inspection of the abdominal cavity. From this array of facts one must conclude that while vaginal hysterectomy is a procedure that must be part of the surgical repertory of every gynecologist it is limited in its applicability. However, the disadvantage of the abdominal complete operation impels us to develop a technic that will facilitate the procedure and reduce to a minimum the disadvantages encountered. Goodall has tabulated the disadvantages of abdominal complete hysterectomy as follows:

1. Greater time expended at operation than for supravaginal hysterectomy.
2. Greater skill required.
3. Greater blood loss.

4. Greater danger to vital organs.
5. Greater difficulty if the pelvic organs are fixed deeply in the pelvic cavity or if the patients are too obese.

An analysis of the above tabulations leads us to the conclusion that the cause of these disadvantages lies in the difficulty of removing the cervix by the abdominal route from its parametrial and vaginal attachments, and also in the difficulty of obtaining proper hemostasis in this region. Even in cases where the cervix is fixed intraabdominally as a result of inflammatory processes, or where adherent retroperitoneal cervical fibroids or adnexal masses are present, the cervix can be more readily

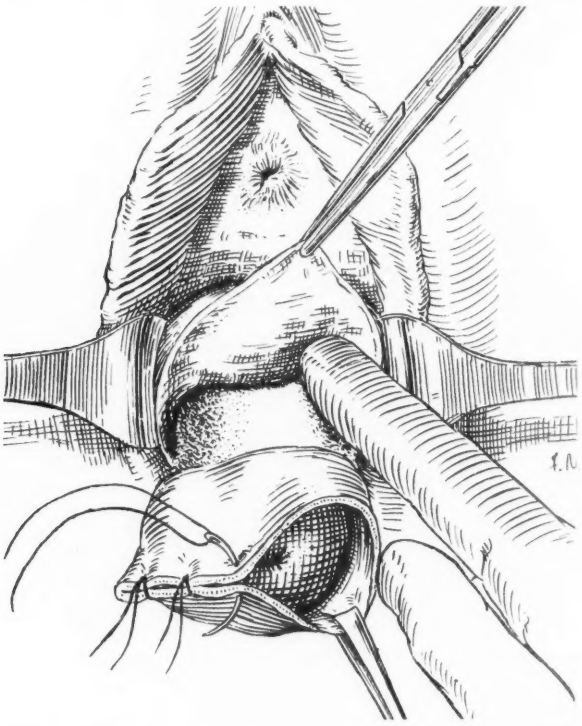

Fig. 1.—Illustrating circular incision about the cervix with mucosal cuff turned down over cervix, and liberation of the bladder from the cervix.

liberated and the parametria and uterine vessels more easily tied by the vaginal than by the abdominal route. It occurred to me that a combined operation would help solve the problem, obviating the difficulties outlined by Goodall and simplifying the procedure so that gynecologists could approach the operation of complete hysterectomy with less reluctance.

TECHNIC

The first stage of the operation is similar to that of a vaginal hysterectomy. The preparation preferred is thorough iodinizing of the vulva, thighs, perineum, and vagina. A circular incision is made circumscrib-

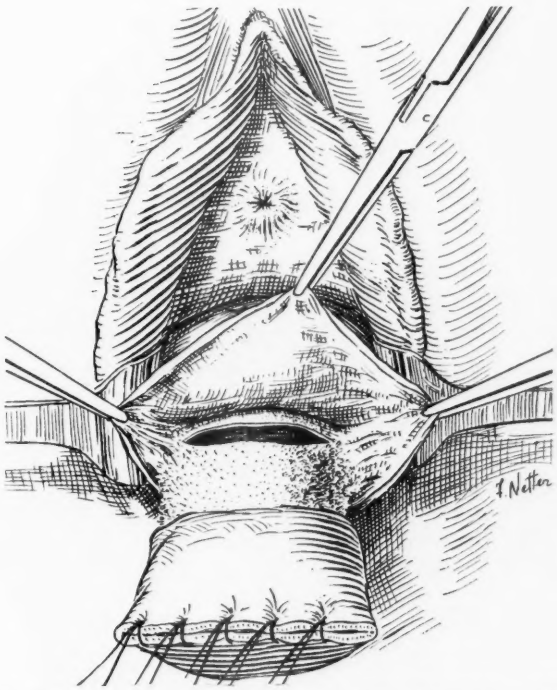

Fig. 2.—Vaginal cuff completely closed, bladder freely liberated. Anterior culdesac incised.

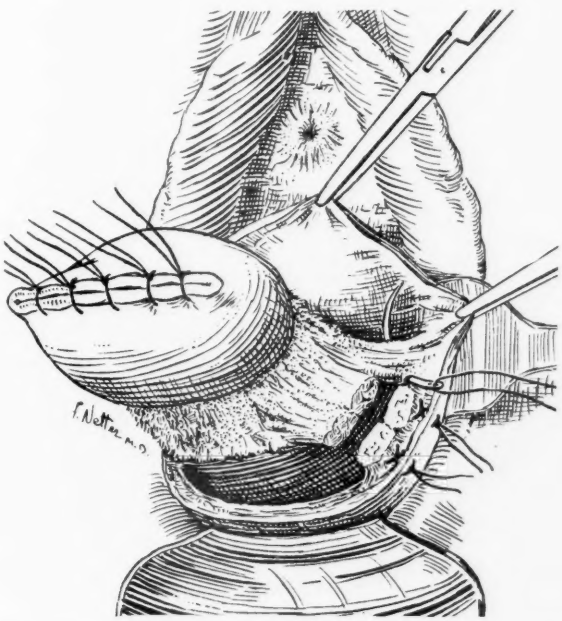

Fig. 3.—Posterior culdesac opened, sacrouterine ligament and parametrial tissue ligated, cut, and anchored to the vaginal wall. Suture placed about uterine vessels.

ing the cervix, leaving a cuff of vaginal mucosa about three-fourths of an inch wide. This is dissected free so that it can be united in front of the cervix with a running stitch thus covering the infected cervix, which must subsequently be delivered through the abdominal cavity. The bladder is pushed upward and the uterovesical peritoneal fold is exposed and opened (Figs. 1 and 2). The posterior culdesac is also widely opened (Fig. 3). Sutures are passed through the posterior portion of the parametrium, one on each side, catching the uterosacral ligament which is cut and freed from the cervix. A second suture is passed through the parametrium above this ligament up to the uterine vessels and the tissue

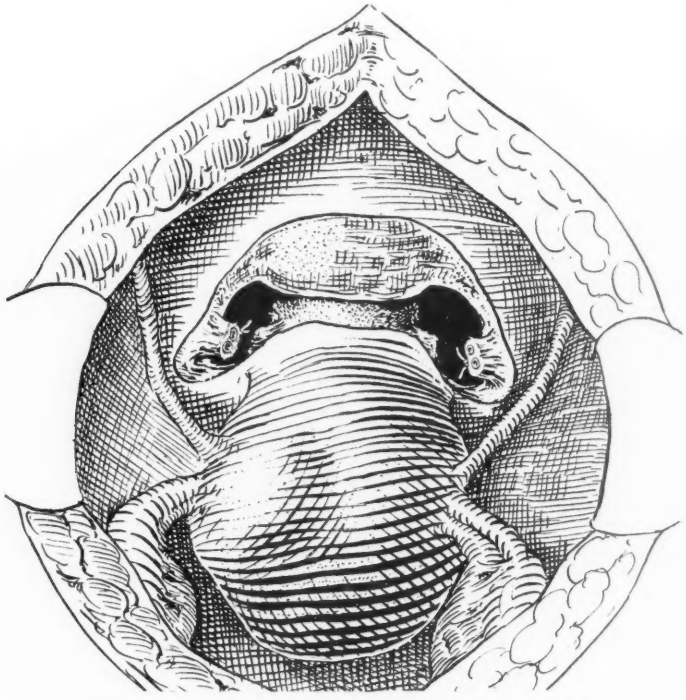

Fig. 4.—Appearance of pelvic floor with the abdomen open after completion of vaginal procedure. Anterior view of uterus showing liberated exposed cervix. The bladder dissected free and the uterine vessels tied.

severed. This may be unnecessary at times if the parametrium is not voluminous. The uterine vessels can now be ligated with either one or two ligatures on each side and cut close to the cervix (Fig. 3). This completes the vaginal procedure except for fixing the parametrial and sacrouterine stumps into the posterior vaginal edge (Fig. 3). The opening in the vaginal mucosa may now be diminished in size by a few interrupted sutures. A large iodoform packing is placed in the vagina up to the everted vaginal mucosal cuff. The patient is now placed in Trendelenburg position and prepared for laparotomy in the routine fashion.

On opening the abdomen the conditions which could not be readily handled if the vaginal operation was continued, such as adhesions, large uterine tumors or ovarian neoplasms, can now be readily taken care of. The infundibulopelvic ligaments are ligated and cut and the round ligaments also. We now find the uterus attached by a few shreds of tissue, usually a small strip of peritoneum on either side and some bundles of connective tissue, i.e., the untied upper portion of the parametrium (Figs. 4 and 5). These are easily cut usually without ligation and the

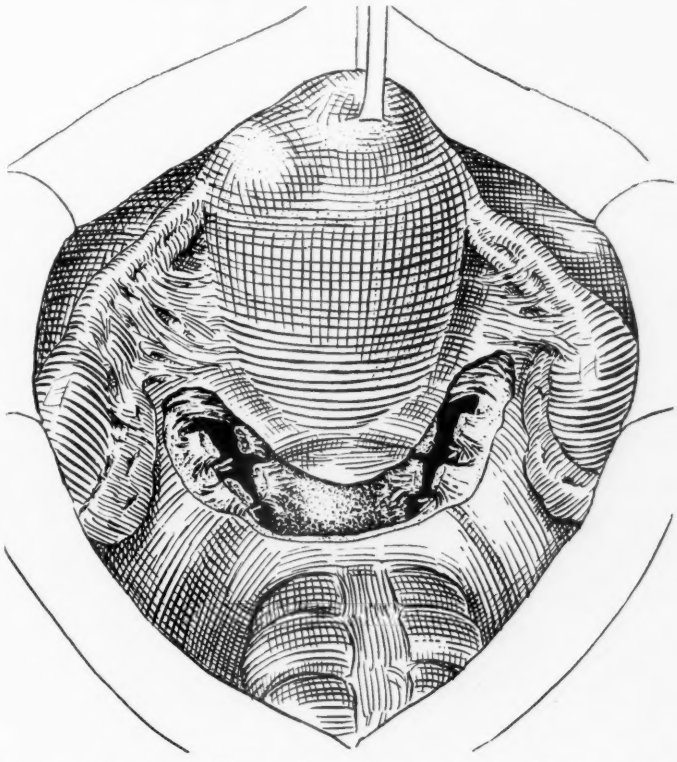

Fig. 5.—Illustrates the condition of the pelvic floor after completion of the vaginal procedure. Uterus drawn forward showing the liberated cervix, the cut and ligated sacrouterine ligament and parametrium.

uterus can be lifted out of the abdomen. The vaginal gauze is removed from below and two narrow strips of iodoform gauze are passed into the vagina on a passer and the abdominal ends of the gauze placed one in each parametrial extraperitoneal space for drainage. If the vaginal opening is still large it can be made smaller by a few sutures. The pelvic peritoneum is closed and the abdominal wall sutured in the usual fashion.

This method makes the access to the uterine vessels simple when an involved inflammatory case or a retroperitoneal or intraligamentous growth prevents easy exposure. The additional time required is negli-

gible. The combined operation which I have had the opportunity of doing only twice took but little more than one hour in both instances. In one case the patient had huge fibroids, one cervical in situation, and in the second case bilateral diseased adnexa adherent to the Douglas peritoneum were present. It is even possible when the posterior vaginal mucosa is incised vaginally to free such growths more readily than from the abdominal side. While the procedure seems to me to offer much, my experience is so limited that I must ask other gynecologists to try it, improve it, and I hope confirm my belief in its advantages.

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UTEROTUBAL INSUFFLATION IN THE MACACUS RHESUS

A Method of Assaying Pharmacologic and Hormonal Effects on Tubal and Uterine Contractions. A Preliminary Report

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PROBLEMS essentially mechanical and physiologic which have arisen during uterotubal insufflation in women appear to be capable of elucidation by studying the results of the application of the same procedure in the monkey. The experimental solution of the physiologic problems depends upon whether contraction waves of the intact uterus and tubes of the monkey can be recorded on the kymograph in a way similar to their clinical demonstration and upon the comparison of these waves with contractions exhibited by the surviving excised genital organs. If this is possible, it should provide a method of assaying in vivo physiologic effects of certain oxytocic, antispasmodic and other pharmacologic substances commonly employed in clinical medicine.

The present study was undertaken to determine whether this method of investigation is feasible and also to note possible changes in rate and amplitude of the tubal and uterine contractions at various stages of the cycle. Incidentally it was felt that certain phenomena observed in the human during uterotubal insufflation might also be checked and possibly explained.

At the outset we were confronted with finding the most practical method of exposing the cervix. Dickinson's observations from a study of thirty adult monkeys showed that the human and simian portio vaginalis bear marked similarities. For cervical inspection alone, the use of Dickinson's modification of the Cushing double-blade nasal speculum sufficed. However, this instrument encroached upon the available space to such an extent as to interfere with the manipulations required for uterotubal insufflation. In order to provide adequate exposure and better to visualize the cervix we resorted to the use of small rectangular, ribbon retractors to draw back the anterior and posterior and also the lateral vaginal walls.

In our experiments the animal anesthetized with nembutal given subcutaneously is placed in the knee-chest position on a triangular block, the cervix is exposed and each lip is grasped by an Allis clamp carry-

ing three or four teeth. The cervix of our parous animals could then be readily pulled downward to the introitus. As was first noted by Sir Arthur Keith and later by Clark and Corner, Dickinson and others, there projects from the anterior or ventral wall of the cervix of the macaque a firm polypoid-like projection, the colliculus. This projection into the cervical canal, as has been emphasized by Dickinson and Engle, offers a definite obstacle to the passage of any instrument and it defeats all attempts at introducing a cannula into the uterine cavity. In order to overcome this difficulty we exposed the colliculus by splitting the cervix bilaterally nearly to the upper border

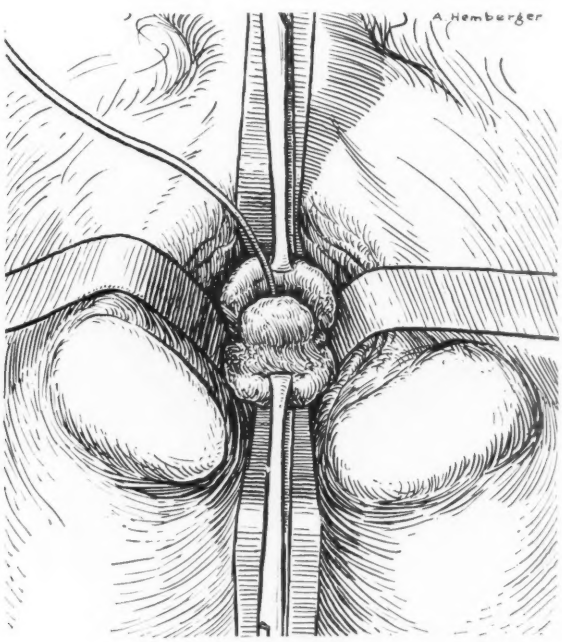

Fig. 1.—Multiparous monkey. Knee-chest position. Cervix drawn downward. Colliculus exposed by retraction of cervical lips following bilateral trachelotomy. Probe indicates course of canal above colliculus.

of the projection. These incisions are associated with surprisingly little bleeding, and so far it has been unnecessary to pass sutures for hemostasis since momentary tamponading has been sufficient to control the relatively slight oozing. Some days after operation, the bilaterally cleft cervix resembles the bilaterally lacerated cervix of the human being and makes possible repeated insufflation without occasioning appreciable bleeding.

In the first monkey operated upon in this fashion a tampon was inserted into the vagina and was retained for several days. This prevented the adhesion of the raw surfaces so that a subsequent introduction of the cannula for insufflation was accomplished with rela-

tive ease. In the next experiments a bilateral trachelotomy immediately exposed the upper border of the colliculus. A fine probe which was then introduced showed that, in the animals employed, the length of the uterus from the external os to the fundus varied from 4½ to 5½ cm. The length of the body was a little greater than that of the cervical canal. A somewhat coarser probe was next passed to dilate the uterine opening sufficiently to permit the insertion of the uterine cannula. The preliminary dilatation we have found to be unnecessary through the procedure of grasping the colliculus with a tenaculum and pulling it downward. This straightens out the canal and exposes the opening which is easily entered by the insufflation cannula. The procedure has been adopted as routine and facilitates the experimental work considerably.

The cannula used is about one-third the width of the instrument commonly employed in the human female, and has ample perforations in the tip to permit the free outflow of gas. The tip of the cannula is introduced within the uterine cavity for a distance of not more than 1½ to 2 cm., the rubber or metallic acorn expansion serving to maintain an air-tight adjustment. The apparatus for insufflation is the same as that employed in clinical gynecology and is gauged for a maximum pressure of 220 mm. Hg.*

An additional obstacle to the experimental project was the resistance encountered at the uterine ends of the tubes. It was found necessary to employ in some instances a pressure level of 200:220 mm. Hg several times, and each time to maintain the high pressure for several minutes, before the uterotubal tone was reduced sufficiently to allow the gas to pass into the tubes and peritoneal cavity. In one instance twenty-two attempts were made within a half hour before success was attained. This time interval may be materially shortened by allowing the pressure to rise up to 300 mm. Hg with a slow rate of insufflation, when the gas may succeed in passing through the tubes, whereupon the pressure drops as rhythmic contractions are recorded on the kymograph. The resistance at the uterotubal junction has been found to be high in all phases of the cycle except possibly near the time of ovulation. This still remains a matter for further study, since our experience is as yet limited to a few monkeys.

When the gas passes through the tubes, rhythmic contractions begin to be recorded upon the revolving drum. They are absent during the initial rise of pressure while the gas is still confined to the uterine cavity and do not appear until the uterine ostiae of the tubes have been passed. While the uterus is increasingly distended, it does not exhibit rhythmic contractions. The latter appear only when the uterus

*The apparatus used in these experiments was made by the Grafax Instrument Company, New York.

is lightly or very moderately distended. The gas thus takes the place of an elastic balloon by which pressure oscillations within the uterine cavity can be recorded.

When tubal contractions are exhibited, the animal is suitable for further experiment with pharmacologic and other substances. When tubal contractions are not demonstrable, owing to almost occlusive resistance at the uterine end of the tubes, the uterine contractions

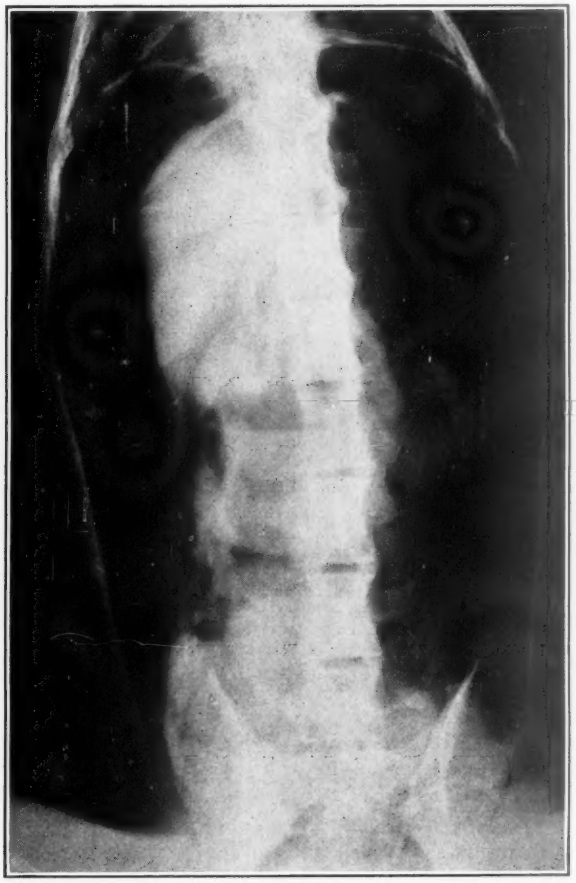

Fig. 2.—Animal 390, showing the distention of the peritoneal cavity by gas introduced by tubal insufflation.

may serve the purpose of the assay. In this preliminary report, we are not prepared to enter into details on this point. However, the effects so far observed from the use of pituitrin and pitocin are prompt and striking.

All the animals which we have employed have tolerated the insufflations well, as much as 2½ to 3 liters of CO_2 gas having been introduced without injury. Following their recovery from the anesthesia, the animals leap about their cages in a typically normal way. In

two animals a laparotomy enabled us to see that the peritoneum was absolutely free of any traumatic or pathologic change, although the abdominal cavity had been distended to drum tightness by 2 to 3 liters of CO_2 gas.

The photographs of Animal 390, taken under the supervision of Dr. Hugh Wilson of the Department of Radiology, show in the first place the distention of the peritoneal cavity which results from the accumulation of gas introduced by way of the tubes, and second, the pneumoperitoneum immediately following the release of most of the contained gas by the insertion of a trocar through the abdominal wall.

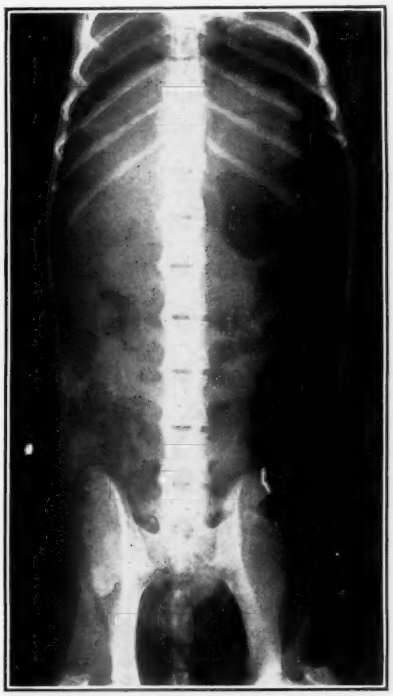

Fig. 3.—Animal 390, showing the peritoneal cavity immediately following the release of gas by a trocar inserted through the abdominal wall.

Further experience has indicated the advisability of inserting the trocar for deflation shortly after the gas begins to enter the abdominal cavity. Thus the experiment respecting tubal contractions may be continued without fear of embarrassing respiration. However, if the rate of introduction of gas is sufficiently slow, it may be unnecessary to resort to coincident deflation since the gas is absorbed very rapidly. This rapidity of absorption was demonstrated by repeated x-ray examinations of one of our animals in which the peritoneal cavity, tensely distended at the beginning, showed an almost complete disappearance of the gas at the end of one hour. When uterine

contractions are to serve as the basis for estimating pharmacologic effects, only a minute quantity of CO_2 gas is required—sufficient to fill the uterine cavity under pressures of 60 mm. Hg or less but not enough to produce a pneumoperitoneum.

In the present report, we wish merely to call attention to the method which has been outlined and which within certain limits appears to offer possibilities for experimental purposes along the lines indicated.

We are indebted for assistance to our technicians, Mr. Joseph Negri and Mr. Frank Caruso. The study was subsidized by a grant from the Research Fund of the Yale School of Medicine.

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BIOLOGIC ASSAY OF ESTROGENIC FACTORS IN PREGNANCY URINE

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PREGNANCY urine has long been known to contain large quantities of estrogenic substances. Commercially, pure crystalline "estrins" are prepared from such urine. The estrins in pregnancy urine are found in a free and combined state as ketohydroxyestrin (theelin and estrone) and trihydroxyestrin (theelol and estriol). These differ not only in their chemical structure but also in their quantitative biologic activity as well. The free "estrins," according to the conception of Marrian, are those that can be extracted readily from urine with ether. The combined "estrins" are not ether soluble and are extractable with this reagent after hydrolysis of the urine either by acidification and boiling or by means of bacterial action which occurs when urine is allowed to stand at room temperature.

In this study no attempt had been made to separate the estrones from the estriols. It covers the biologic assay of the total estrin output in one case of a normal pregnancy from the ninth to the forty-first week and the relationship of the free to the combined estrins in 35 normal pregnant women at different periods of pregnancy.

METHOD

1. Chloroform extracts of bacterially hydrolized pregnancy urine samples were titered by the Allen and Doisy method in the castrate mouse.

2. Nonextracted pregnancy urine specimens, after bacterial hydrolysis, were titered as in Method 1.

3. Chloroform extracts of chemically hydrolized fresh pregnancy urine was titered as in Method 1.*

4. Ether extracts of 35 fresh twenty-four-hour pregnancy urine specimens were titered as in Method 1.

5. Assay of the same urine as used in Method 4 after bacterial hydrolysis.

RESULTS

The continuous study of a normal pregnancy (Chart 1) showed that the total estrogenic output rose slowly from the ninth to the twenty-ninth week from 1,000 M.U. to 20,000 M.U. in the twenty-four-hour specimens examined weekly. Comparing this with the international unit standard, this rise is equivalent to the difference between $\frac{1}{10}$ mg. and 2 mg. of crystalline "estrins." On the twenty-eighth week, antepartum, there was a sudden rise in estrogenic factors to 125,000 M.U. in nonextracted urine and 119,000 M.U. by the chloroform method. The high estrogenic

*Method 3 was carried out for comparison to determine if bacterial hydrolysis is complete.

output after the twenty-ninth week was maintained, reaching as high as 180,000 M.U. or the equivalent of 18 mg. of crystalline hormones up to the thirty-third week, when a drop to 43,750 M.U. occurred.

It rose again during the thirty-fourth, thirty-fifth, and thirty-sixth weeks to 160,000, and then dropped in the thirty-seventh, thirty-eighth, thirty-ninth, and fortieth week specimens to 39,000, 45,000, 45,000, and 11,200, respectively. Forty-eight hours postpartum, the output averaged 20,000 M.U.

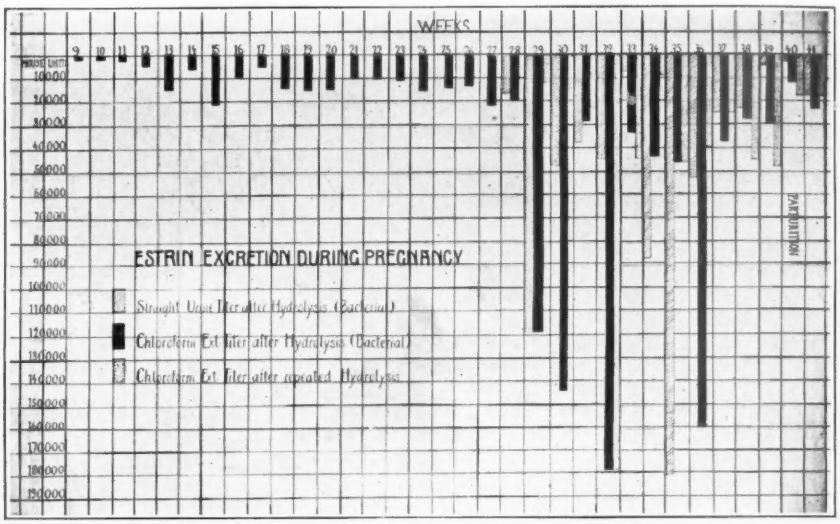

Fig. 1.

Fig. 2.

The 35 twenty-four-hour specimens assayed at different periods of pregnancy from 35 different women showed that there was a marked variation in the percentage relationship between the free and the combined "estrins" (Chart 2). The free estrins represented from 2 per cent to 22 per cent of the total daily output of active substance. When the total free estrins are compared with the total combined estrins there is an increase in the last eight weeks of pregnancy (Chart 3).

DISCUSSION

Ever since pregnancy urine has been found to contain estrogenic hormones, discrepancies in titer became apparent. Marrian has been

able to influence the titer by hydrolysis. This may be accomplished equally well by acidification or bacterial action (allowing urine to stand at room temperature for several days).

Cohen, Marrian and Watson have studied the excretion of the estrogenic factors during pregnancy on samples obtained from 20 different pregnant women. Their method of assay was a chemical one, namely, a modification of Kober's colorimetric assay method. They found that the "estrin" excretion rose rapidly at about the third month, maintained a high level throughout the remainder of the pregnancy, and finally fell to a low level a few days before or at delivery.

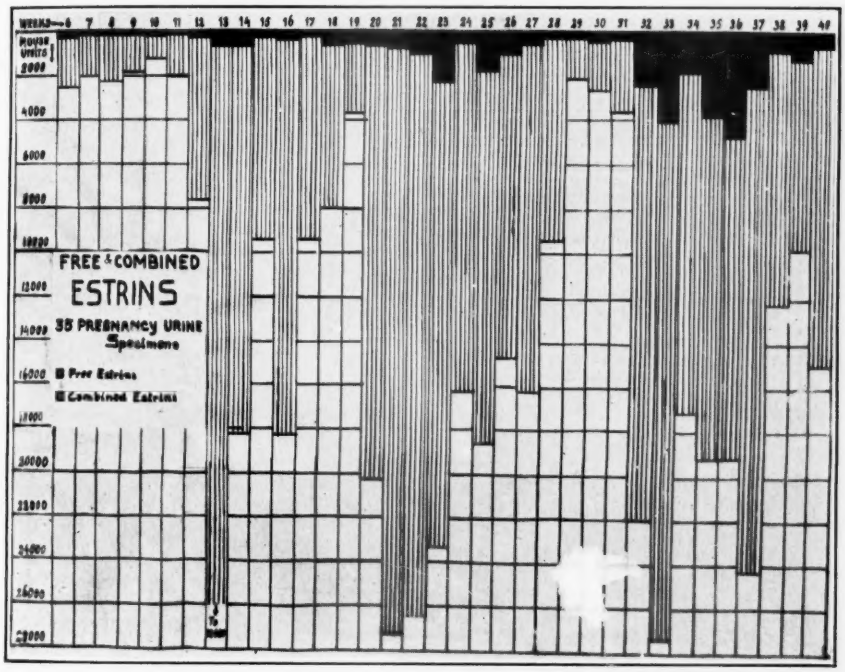

Fig. 3.

From estimations on the quantitative excretion of free and combined estrins and estriols, they concluded that 99 per cent of the total estrogenic factors found in pregnancy urine up to the eighth month were in the combined ether-insoluble form, and, as they interpreted it, physiologically inert. This, they attributed to the ability of the pregnant woman to inactivate estrin. They also suggested that the onset of labor is dependent upon a drop in the combined estrins and a rise in the free forms with consequent contractile effect on the uterine muscle.

The finding of 99 per cent of the estrogenic factors in the combined ether-insoluble form, as reported by these authors, seemed so greatly at variance with our experience in the assay of fresh and chloroform ex-

tracts of urine by means of biologic methods, that it was deemed essential to determine whether biologic assays would show a similar relation.

The biologic assay in this study shows that the total estrins excreted in normal pregnancy is low up to the twenty-ninth week and highest between the twenty-ninth and thirty-sixth weeks. It again drops to a low level in the last four weeks of pregnancy. The free estrin excretion found in this investigation was greatest between the thirty-second and fortieth weeks.

The percentage of free estrins varies from 2 per cent to 22 per cent and shows no definite relationship to the period of pregnancy. Before drawing definite conclusions a large series of cases should be investigated.

CONCLUSIONS

1. There is an increase in the combined estrin excretion as pregnancy advances. This reaches its highest point between the twenty-ninth and thirty-sixth weeks.

2. There is a diminished excretion of combined estrins in the last four weeks of pregnancy.

3. The free estrin excretion is greatest between the thirty-second and fortieth weeks.

4. No constant percentage relationship is noted between the duration of the pregnancy and the excretion of free estrins.

5. Assays of pregnancy urine following bacterial hydrolysis are concordant with those obtained with acid hydrolysis.

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NOTE

For lack of space it is not possible to include in this issue of the JOURNAL *all of the papers contributed to the Robert T. Frank Anniversary Number. Several will appear in the July number and all will be published in a special volume to be issued at a later date by the committee in charge.*

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